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INSTRUCTIONS



# Emulsion Polymerization of Synthetic Rubber in 10-Gram Systems

## An Experimental Technique

CHARLES F. FRYLING, The B. F. Goodrich Company, Akron, Ohio

The experimental procedure consists of sealing the ingredients of a polymerization recipe into a test tube and rotating the tube at a constant temperature, following the course of the reaction by noting the decrease in volume of the system. The latex is removed for coagulation when the polymerization has proceeded as far as desired, and the yield is determined by weighing the dried stabilized coagulum.

IN DEVELOPING practical recipes for the manufacture of synthetic rubber, it was desirable to investigate the effects on the polymerization process of a large variety of highly purified substances. Only small quantities of many materials were available. Furthermore, in order to obtain valid comparisons between experiments conducted over a period of time, it was necessary to keep standardized samples of the major components and to use them as economically as possible. These considerations led to the development of a small-scale polymerization technique whereby from 10 to 20 grams of monomers could be employed. By this method an extraordinary amount of valuable information was obtainable. This included:

1. Yield
2. A polymerization reaction curve from which to estimate length of induction period, if any; rate of polymerization at any desired time; and over-all conversion at any desired time
3. Kind of emulsion—i.e., whether fluid, viscous, gelatinous, or heterogeneous, at any stage in the process
4. pH of emulsion at end of process
5. Qualitative observations on coagulation
6. Production of a sample of synthetic rubber sufficiently large to determine solubility, milling characteristics, and cured properties by procedures described by Garvey (2)

This small-scale technique has been employed for investigating polymerization of a large number of monomers and comonomer mixtures, for evaluating emulsifying agents, for determining the effect of impurities in the reagents employed, for varying the comonomer ratio and the ratio of hydrocarbons to aqueous phase, and for investigating behavior of various initiators, inhibitors, and other ingredients of the polymerization recipe. It proved to be especially advantageous in providing information on the effect of some one ingredient over a range of concentrations. The influence of reaction temperature was readily determined. In general, the advantages of the technique were particularly apparent in:

Preliminary surveys, where wide areas of investigation had to be covered in the shortest possible time. The method is amenable to simple labor-saving tricks, such as filling a large number of reaction tubes at the same time with solutions of a given emulsifying agent.

Control testing of raw materials. The particular properties essential for polymerization of all shipments of materials intended for large-scale production can be tested quickly. A good correlation can be obtained between the experimental results of this method and behavior on a manufacturing scale.

The most serious limitation to this technique is that materials cannot be added to or subtracted from the system once polymerization has started. [Balandina *et al.* described a similar technique, the details of which are not readily available to English-speaking investigators (1).]

### RECIPES FOR POLYMERIZING SYNTHETIC RUBBER

The development of satisfactory polymerization recipes is one of the important objectives of synthetic rubber research. Patent literature contains many examples of such. The following, from a U. S. patent issued to Wollthan and Becker (5), and recalculated to the scale of this technique, is perhaps typical:

Butadiene	7.5 grams
Styrene	2.5 grams
Isohexyl mercaptan	0.05 gram
Water	18.0 grams
Sodium oleate	2.0 grams
Ammonium persulfate	0.03 gram
Temperature	30° C.
Time	"Several days"
Yield	"Excellent"

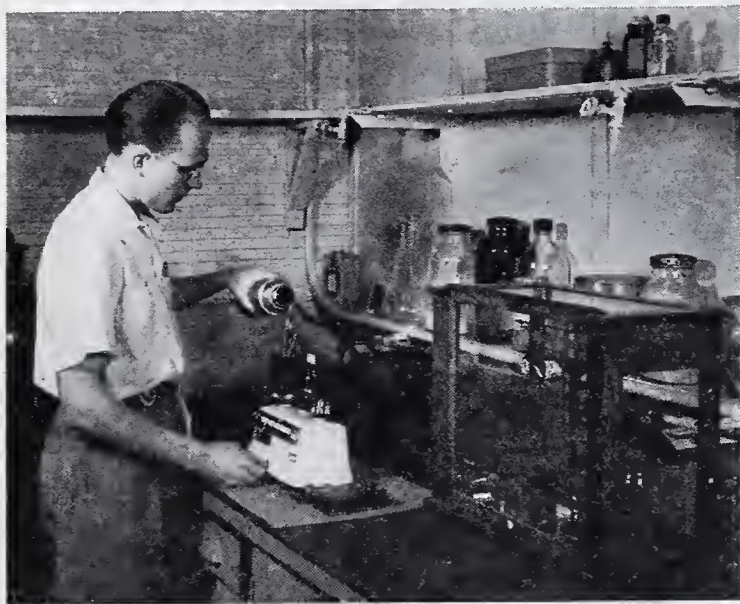
An understanding of the function of each ingredient is essential. In the above example, the butadiene and styrene are the monomers, which, by copolymerizing, form synthetic rubber. The isohexyl mercaptan is described as a substance exerting a "regulating effect"—i.e., it possibly decreases the branching characteristics of the resulting polymer. Sodium oleate is the emulsifying agent, and the ammonium persulfate acts as the polymerization initiator, also called the "polymerization catalyst".

The possibilities of research on such a system are great and are increased by the fact that a variation introduced in any one ingredient may require a concomitant change in some other ingredient. For example, substituting another substance for the initiator might require a change in the type of emulsifying agent employed in order to get satisfactory results.



## GENERAL CONSIDERATIONS ON TECHNIQUE

Polymerization, while a science, is also an art. The way in which things are done—that is, the niceties of experimental technique employed—is of equal importance to the scientific aspects of the subject. Unless this viewpoint is kept clearly in mind, the investigator is frequently confronted by baffling failures. Emulsion polymerization is particularly susceptible to the influence of traces of contaminants. The equipment of a research laboratory may be covered with dust which contains inhibitors or accelerators of polymerization, indeed, some substances, which under certain conditions inhibit polymerization, may under slightly different conditions act as catalysts. Nevertheless, the difficulties confronting the investigator can be avoided with a little care and forethought.



Weighing Butadiene

In general, solutions or other substances to be employed in polymerization experiments should not be exposed to atmospheric contamination for longer periods than necessary. Glass stoppers afford adequate protection. The contents of a flask may be temporarily protected by covering the mouth with a sheet of clean dry tinfoil. Cork stoppers should be avoided; but if necessary, they can be covered with tinfoil. In no case should the alkaline contents of flasks come in contact with tinfoil.

Glass bottles and flasks must be clean. In most cases treatment with chromic acid cleaning solutions, followed by rinsing with tap water and then distilled water, is adequate. Reaction tubes, however, require more effective cleaning.

In one operation it is convenient to pour the volatile contents of a Dewar flask through a short length of rubber tubing. Although rubber contains antioxidants, accelerators, and other chemicals, no trouble is experienced from this source if the rubber tubing is first extracted by boiling in several changes of acetone. This can be done (on a steam plate) in a covered beaker if a sizable piece of dry ice is placed on the watch crystal, which thereby becomes a convenient reflux condenser. The extracted tubing can be kept in a stoppered wide-mouthed bottle for future use.

Certain monomers can be efficiently separated from powerful inhibitors added as stabilizers by distillation through relatively simple equipment. The practice of distilling monomers in the absence of an inhibitor should be avoided because of the danger of explosions due to the accumulation of peroxides in the distillation residue.

It has been customary in the laboratory to prepare fresh samples of butadiene by condensation in clean glassware from a stream of gas taken from a large cylinder of liquefied material. Higher boiling monomers are freshly prepared by atmospheric, vacuum, or steam distillation as required. All-glass distillation equipment is most satisfactory. The unstabilized monomers can be kept stoppered in a refrigerator at  $-30^{\circ}\text{C}$ . for several days without detectable deterioration.

Sometimes repetition of an operation is advisable. Metallic

polymerization vessels, no matter how carefully cleaned, may be inhibited; merely emptying and recharging are often sufficient to ensure a satisfactory reaction.

There is no substitute for constant care and cleanliness on the part of the investigator. A careful experimenter can easily add just the weight of small portions of certain monomers using a clean medicine dropper, while a careless experimenter (performing the same operation) can ruin a large number of experiments by allowing the monomer to come into contact with the rubber bulb of the medicine dropper.

## EXPERIMENTAL PROCEDURE

The ingredients of a polymerization recipe are sealed into a test tube, which is rotated at a constant temperature. The course of the reaction is followed by noting the decrease in volume of the system, and the latex is removed for coagulation when the polymerization has proceeded as far as desired. The yield is determined by weighing the dried, stabilized coagulum.

Pyrex reaction tubes, 22 mm. in diameter, approximately 50 ml. in capacity, to the upper end of which are sealed 10-mm. diameter Pyrex tubes, may be obtained in gross lots from the Corning Glass Company, according to the following specifications: "Glass tubes, Pyrex, 22 mm. O.D.  $\times$  1.5 mm. walls (uniform), 215-mm. body to neck, 22-mm. tapered shoulder, 145-mm. neck. 10-mm. neck  $\times$  1-mm. wall thickness". These can be made by the experimenter, but it has been found cheaper to purchase them.

New tubes are cleaned by rinsing with distilled water and anhydrous c.p. synthetic methanol, in that order, and evacuating until dry. Evacuation may be accomplished with a Cenco Hyvac pump connected in train through a dry ice-acetone trap which condenses the methanol and prevents diffusion of any volatile inhibitor back into the tube. Acetone-extracted rubber tubing is used to attach the reaction tubes to the vacuum system.

Old tubes, after being cleaned in a chromic acid bath, are repaired by sealing new necks of 10-mm. Pyrex tubing, 10 cm. long, to the shoulder of the tubes. The open ends are fire-polished. The tubes are then subjected to the cleaning treatment recommended by Suess, Pilch, and Rudorfer (4). Clean concentrated nitric acid is poured into the tubes and allowed to stand from 16 to 24 hours. If the tubes are required at once they are filled to the shoulder with nitric acid and gently heated from 15 to 30 minutes, then rinsed three times with tap water. One rinse should completely fill the tube to displace all the fumes. After two additional rinses with distilled water, the tubes may be dried with synthetic methanol and evacuated. If desired, the methanol rinse and evacuation can be dispensed with by allowing the distilled water to drain from the inverted tubes overnight. The dry tubes are stoppered with No. 0 corks, which have been covered with fresh tinfoil, and the tubes are kept in a clean place until used.

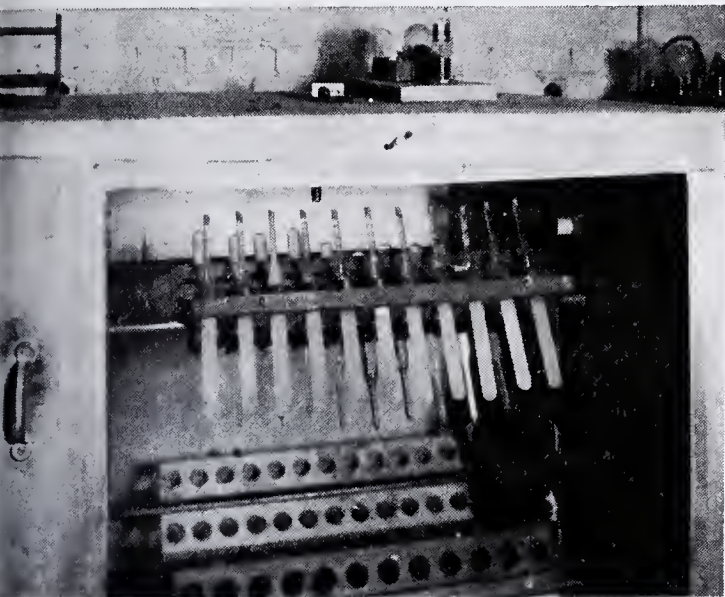
Solids may be added to the reaction tubes in quantities as low as 0.5 mg. from small aluminum foil weighing scoops. If dupli-



Adding Butadiene to Reaction Tube



ate quantities are to be used throughout a series of experiments, it is convenient to dissolve organic compounds in the less volatile monomer and inorganic compounds in the aqueous solution of emulsifying agent.



Rotation of Tubes in Constant-Temperature Cabinet

The solution of emulsifying agent is generally prepared separately and added to the reaction tubes from a pipet or a graduated cylinder. Since slight variations in the ratio of monomers to aqueous phase have little effect, convenience generally dictates the use of the latter method. The volume of emulsifying agent employed may vary from 10 to 30 ml. for 10 grams of monomers.

The reaction tubes containing the emulsifying agent and other ingredients are placed in a refrigerator, inclined at an angle of 2° 8' from horizontal, and frozen at -30° C. This inclination may be obtained by placing a piece of 10-mm. glass tubing under the necks of the tubes. Unless chilled in this position, the tubes will crack when placed in a dry ice-acetone bath. (The cork-stoppered glass reaction tubes may be laid on a table at the proper inclination and covered with dry ice. However, any contamination of the contents by carbon dioxide will alter the pH of the soap solution and affect the reaction rate seriously.) If care is exercised, the aqueous phase can be frozen by direct immersion of the tubes in a dry ice-acetone bath, with frequent withdrawals and nearly horizontal rotations, so that the aqueous phase freezes in contact with the glass in the form of a hollow cylinder. The losses due to cracking of the glass are high and the operation is time consuming; therefore the method of freezing first described is preferred in most cases.

In the next step the frozen tubes are individually cooled further in a dry ice-acetone mixture contained in a quart-size straight-sided Dewar flask. A rubber dam is fitted over the tube and the Dewar flask, the neck of the tube extending through a hole in the dam. This minimizes contamination of the tube with escaping carbon dioxide, and holds it in a convenient vertical position.

The higher boiling comonomer is weighed to 0.1 gram into a tared, clean microbeaker and poured into the reaction tube. A buret is sometimes used for this operation but contamination by stopcock grease should be avoided. In either case the operation must be performed in a rigorously clean manner.

Freshly distilled butadiene is temporarily contained in a pint-size Dewar fitted with a two-hole extracted rubber stopper through which extend two short glass tubes arranged for convenient pouring. The temperature of the butadiene is held at -30° C. It is weighed to 0.1 gram into a small silvered Dewar weighing flask using a torsion balance. The small Dewar is then attached to the reaction tube by a short length of extracted rubber tubing and the butadiene is poured into the reaction tube, allowing about 45 seconds for condensation of vapor before removing the rubber tube. The weighing flask has a 10-mm. neck, and is 11.5 cm. from bottom to shoulder, 35 mm. in outside diameter, 25 mm. in inside diameter, and about 16 cm. in over-all length. Such flasks have been made in the laboratory but it has been found more satisfactory to have them made by professional glass blowers.

The reaction tube is sealed, using a hand blow torch. It is ad-

visible to do the sealing close to the open end of the neck in an oxidizing atmosphere; otherwise a carbon mirror may form on the interior surface of the tubing and prevent a tight seal. Occasionally, if condensation is not complete, a slow blue flame travels from the heated glass down into the reaction tube. It has been impossible, however, to demonstrate that this brings about any variation in the ensuing polymerization.

POLYMERIZATION

The sealed reaction tube is brought to reaction temperature by immersion in water. The height of the meniscus is determined and recorded, using a millimeter rule. The tube is then rotated at a constant temperature and readings of the meniscus height are made periodically. It is from these readings that reaction curves such as Figure 1 can be plotted.

If the polymerization is complete in less than 5 hours, a water thermostat is required to prevent temperature buildup. However, a thermostatically controlled air cabinet, provided with shafts for rotating the tubes, is more convenient.

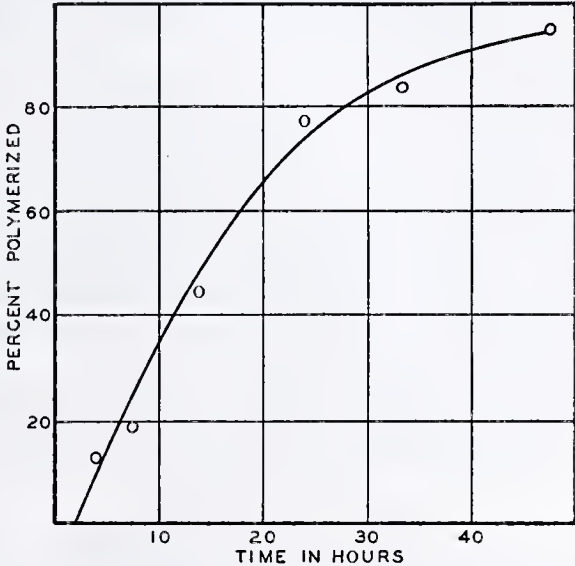


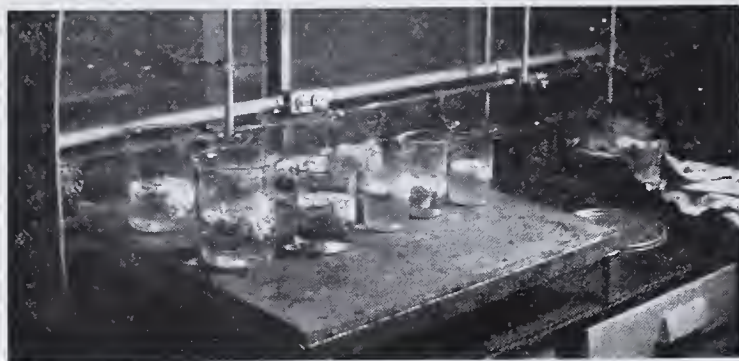
Figure 1. Polymerization Curve

A reaction tube, prepared as described, using the Wollthan and Becker recipe (5), will give an initial meniscus height of approximately 110 mm. During the course of polymerization, it will drop 11 mm. Since the height can be read to 0.5 mm., this procedure provides a simple method of following the reaction rate with an accuracy of about 5%. Table I shows for other recipes that the drop in height of the meniscus is directly proportional to the yield of polymer, within the limits of error of the method. If the total decrease of meniscus height is measured just before opening the tube, the yield, as measured on the dry polymer, will

Table I. Correlation between Percentage Polymerized and Fall of Meniscus for Three Polymerization Recipes

	Fall of Meniscus <i>Mm.</i>	Yield		Difference %
		Measured %	Calculated from meniscus %	
Series I	3.0	18	19	+1
	7.0	39	44	+5
	10.4	61	65	+4
	14.1	89	89	...
Series II	3.0	21	19	-2
	7.0	43	45	+2
	10.9	70	70	0
	14.4	93	93	...
Series III	2.0	9	13	+4
	4.0	20	26	+6
	4.5	26	29	+3
	5.8	38	37	-1
	7.6	44	49	+5
	7.8	47	50	+3
	11.0	67	70	+3
	12.6	86	81	-5
	14.7	94	94	...





Washing Coagulated Synthetic Rubber Samples

give an accurate basis for calculating the partial yields at any given time during the process. Reaction curves plotted using these figures will not be affected by errors due to variations in diameter of the individual tubes.

The formation of foam, which breaks with difficulty, frequently interferes with the measurement of meniscus height. In such a case the tubes can be centrifuged by swinging in a suitable tube on the end of a 90-cm. (3-foot) rope. [According to Reynolds (3) the accuracy of this method can be improved by constriction of the tube at the position where the meniscus is read, together with high-speed centrifuging and redispersion of the emulsion by shaking after the reading.] If a gel forms, or if there is much coagulation during polymerization, the height of the meniscus cannot be determined accurately.

The end of the induction period, or the beginning of polymerization, is generally indicated by the appearance of a bluish opalescence, in addition to the change in height of the meniscus.

#### DETERMINATION OF YIELD

Opening the reaction tubes presents no difficulty except when low partial conversions are under investigation. Then it is nec-

essary to break the tip and direct the violently expelled foam into a large beaker. The synthetic rubber latex must next be stabilized by the addition of an antioxidant. Two per cent of dispersed phenyl-beta-naphthylamine has been found convenient and satisfactory. The dispersion of the stabilizer can be obtained by aqueous dilution of an alcoholic solution. The latex can be coagulated by any method customarily employed for breaking emulsions or coagulating natural rubber latex. Following coagulation, the rubber is washed free of soap and electrolytes, using a Büchner filter and filter paper, and dried in air, preferably at a low temperature. The yield, accurate to  $\pm 2\%$ , can be obtained by weighing to 0.1 gram.

#### SUMMARY

Many manufacturers of monomers, emulsifying agents, initiators, modifiers, and other ingredients going into polymerization reactions find it necessary to have a reliable polymerization procedure for testing the quality of their products. The procedure presented, despite some shortcomings, has many advantages. Minimum amounts of material are required and large numbers of experiments can be conducted in a relatively short time. A serious effort is made to point out some of the pitfalls which beset investigators of polymerization regardless of the type of technique employed. The best recommendation for the procedure described is that it has been used to develop certain types of synthetic rubber which are now in commercial production.

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- (4) Suess, Pilch, and Rudorfer, *Z. physik. Chem.*, A179, 361- (1937).
- (5) Wollthan and Becker, U. S. Patent 2,281,613 (May 5, 1942).

PRESENTED before the Division of Rubber Chemistry at the 105th Meeting of the AMERICAN CHEMICAL SOCIETY, Detroit, Mich.

## Determination of Tetraethyllead in Gasoline

HARRY GONICK AND J. J. MILANO, Shell Oil Company, Incorporated, Martinez, Calif.

A method for the determination of tetraethyllead in gasoline is described in which the tetraethyllead is decomposed with iodine and the lead subsequently titrated by a new acidimetric method employing 8-hydroxyquinoline. The method is rapid and is applicable to all types of gasolines.

UNTIL recently the most widely used method for the determination of tetraethyllead in gasoline was the bromination method described by Edgar and Calingaert (3) in which the tetraethyllead was decomposed by the action of bromine. Although this method was rapid and convenient for the determination of tetraethyllead in straight-run gasolines, difficulties were encountered with cracked gasolines owing to the rapid absorption of bromine by the olefins present, in competition with the tetraethyllead. With gasolines of high olefin content it was necessary to brominate the gasoline completely to ensure complete decomposition of the tetraethyllead. Even so, low results were frequently obtained. Moreover, the quantity of bromine required for complete bromination of a gasoline of high olefin content was rather large (frequently in excess of 200 grams) and added substantially to the cost of the analysis. In addition, the bromination reaction was violent and was accompanied by the evolution of corrosive vapors which caused considerable hazard to the operator. For these reasons, the

bromination method was not suited to the routine analysis of cracked fuels.

More recently other methods have been devised in which the gasoline is treated with hydrochloric acid and the lead determined in the acid extracts. The best known of these is the method of Calingaert and Gambrill (2), recently adopted by the American Society for Testing Materials as a tentative standard (1). Although this method gives satisfactory results with all types of gasolines, the over-all time required for a determination is somewhat lengthy and specialized equipment is required.

The method described in this paper was developed in an effort to reduce the time required for the tetraethyllead determination. By the proposed method, a single determination can be completed in one hour and for determinations in quantity only a small fraction of this time is required per determination. The accuracy of the method appears to be equal to previous methods and does not appear to be affected by the type or composition of the gasoline. More than three thousand samples of gasoline



representing all the principal brands and grades sold in the western states, have been analyzed successfully.

### PRINCIPLE OF PROPOSED METHOD

As in previous methods, the determination of the tetraethyllead divides itself into two distinct parts: (1) the decomposition of the tetraethyllead to yield an inorganic lead salt, and (2) the determination of the lead.

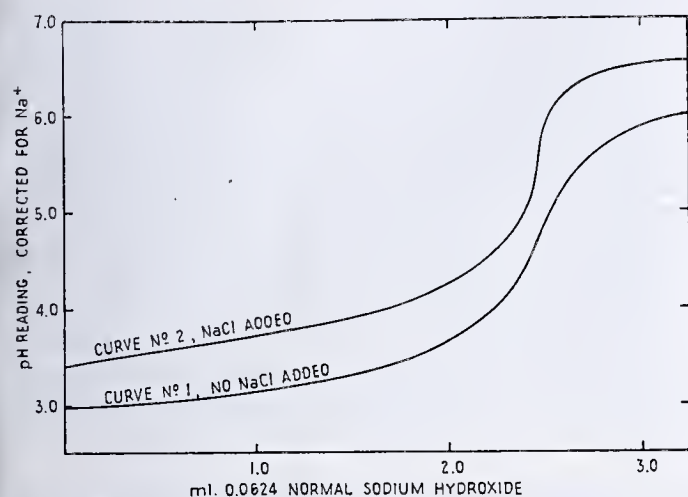


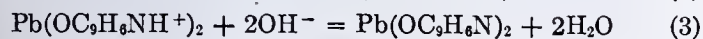
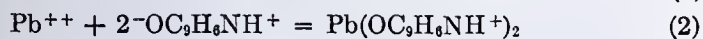
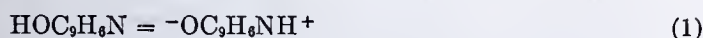
Figure 1. Effect of Sodium Chloride on Neutralization Curve of Solutions Containing Lead Ions

0.137 gram of  $Pb(NO_3)_2$  in 100 ml. of water. (1) No NaCl added, (2) 5 grams of NaCl added

1. Experiments conducted in this laboratory showed that tetraethyllead is rapidly and quantitatively decomposed by the action of free iodine. Unsaturated hydrocarbons do not interfere, as they do not iodinate so rapidly as to compete with the tetraethyllead reaction. The gasoline is removed by evaporation under a hot air stream, and any organic matter remaining is subsequently oxidized with nitric acid and potassium chlorate. The inorganic residue remaining then contains all the lead in the form of inorganic salts including lead iodate, which is insoluble in water. The lead salts so obtained are converted to the more soluble chloride by treatment with hydrochloric acid.

2. The lead is determined by an acidimetric titration method; it is therefore necessary to neutralize the solution exactly before titrating the lead. The neutralization of a solution containing lead salts ordinarily presents difficulties owing to the hydrolysis of the lead. These difficulties are obviated, however, by the presence of sufficient chloride ions which effectively suppress the hydrolysis of the lead. The effect of sodium chloride on the neutralization of a lead solution is shown by the curves in Figure 1.

After the solution has been neutralized, an excess of 8-hydroxyquinoline is added. This reagent reacts with the lead ions to liberate an equivalent quantity of acid which is then titrated with standard alkali. The reactions are assumed to be as follows:



As indicated in Equation 1, 8-hydroxyquinoline is amphoteric and goes over to the ionic form. When 8-hydroxyquinoline reagent is added to a solution containing lead ions, lead 8-hydroxyquinolinium ions are formed according to Equation 2. These are quantitatively titrated with standard alkali to pH 7 according to Equation 3. Other equilibrium reactions are undoubtedly involved, including reaction between lead 8-hydroxyquinolinium ions and excess reagent; however, the equations given indicate the essential result.

### DETAILS OF METHOD

**APPARATUS.** The hot air-jet evaporator (Figure 2) is designed to direct a hot air stream into four Erlenmeyer flasks simultaneously during evaporations. Although the design shown has proved very satisfactory in actual practice, other designs which will accomplish the same purpose may be used.

**REAGENTS.** Iodine, saturated solution in carbon tetrachloride (technical). Nitric acid, c.p., concentrated. Potassium chlorate, c.p., crystals. Hydrochloric acid, c.p., dilute solution; 1 to 1. Sodium chloride, c.p., crystals. 8-Hydroxyquinoline, 0.065N in 60 per cent isopropyl alcohol. Standard sodium hydroxide, 0.0624N. Standard hydrochloric acid, 0.0624N. (Standard 0.0624N acid and base were selected since these reagents are in general use in oil laboratories.)

Methyl red indicator; dissolve 1 gram in 600 ml. of alcohol and dilute to 1 liter with water. Phenol red indicator, 0.2 gram per liter of water.

**SEPARATION OF LEAD.** Measure exactly 100 ml. (corrected to 60° F.) of the gasoline to be tested into a 500-ml. Erlenmeyer flask.

Add 50 ml. of the iodine solution and allow to stand for at least 5 minutes. Place the flask on a hot plate under a hot air stream and evaporate the gasoline to dryness. The velocity of the air stream and the temperature of the hot plate should be regulated so as to secure the maximum rate of evaporation without spattering or bumping. The air stream effectively suppresses the tendency toward bumping which is almost unavoidable without its use. The evaporation ordinarily takes from 15 to 20 minutes.

Add 25 to 50 ml. of concentrated nitric acid (depending on the amount of the organic residue) and rotate the flask over a burner until dense fumes of iodine and nitrogen dioxide cease. Should any organic matter adhere to the walls of the flask continue rotating the flask until it is completely dislodged.

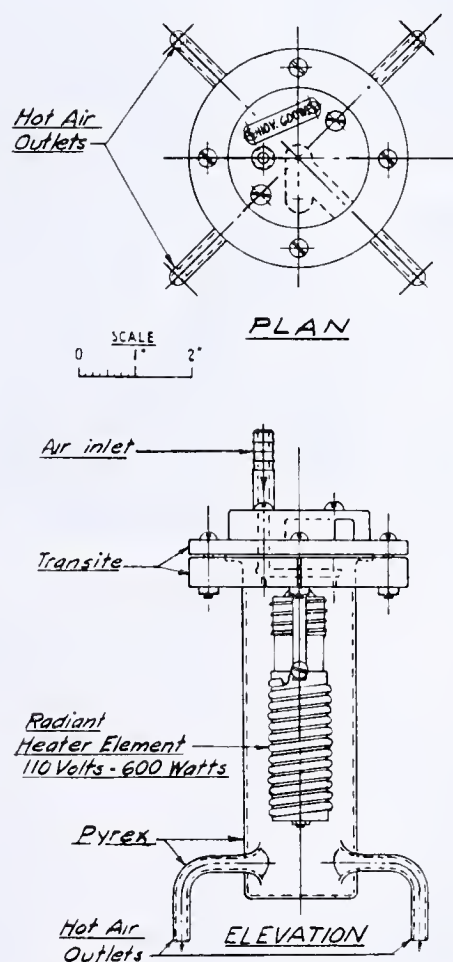


Figure 2. Hot Air-Jet Evaporator

All interior metal parts must be heat-resistant

To the actively boiling solution add crystals of potassium chlorate until the organic matter is completely destroyed. The potassium chlorate should be added cautiously. The solution should not be permitted to evaporate to dryness while there is visible organic matter present as indicated by a brownish coloration of the solution; otherwise spontaneous ignition will occur and cause losses of lead. Usually all organic matter disappears after the first 2 or 3 grams of potassium chlorate have been added; however, one or two additional portions of 2 or 3 grams each are added in order to complete the oxidation. With practice, the oxidation of residues from cracked gasolines with potassium chlorate can be effected in 1 to 3 minutes.

Evaporate the clear nitric acid solution to complete dryness. Should the solution exhibit any darkening during the evaporation,



Table I. Determination of Tetraethyllead in Synthetic Gasoline Blends by the Proposed Method<sup>a</sup>

Samples	Tetraethyllead Content	
	Calculated	Determined Ml./gallon
50% cracked gasoline + 20% straight-run gasoline	3.07	3.07, 3.06, 3.07
	1.54	1.54, 1.54, 1.56
	0.20	0.19, 0.20, 0.19
100% straight-run gasoline	3.00	3.00, 3.00
	1.50	1.51, 1.50
	0.30	0.29, 0.29
50% cracked gasoline + 50% isopentane	1.54	1.54
50% cracked gasoline + 50% alcohol	1.54	1.55

<sup>a</sup> These blends were made using ethyl fluid obtained from the Ethyl Corp.

add more potassium chlorate. The use of the hot air stream is not recommended during this operation, as the cooling effect of the air stream impedes the oxidation of possible traces of organic matter. To ensure the complete destruction of organic matter, heat the residue over a burner until it is completely fused. The residue after fusion should be white (see note below on the use of potassium chlorate).

Allow the flask to cool somewhat, and add sufficient 1 to 1 hydrochloric acid to dissolve the residue completely after 2 or 3 minutes' boiling. Usually 10 to 20 ml. of the dilute acid are sufficient. After complete solution is effected evaporate to dryness. Special care should be exercised toward the end of the evaporation, as the potassium chloride formed has a tendency to spatter. Remove the remaining acid as completely as possible by thoroughly heating the flask while blowing a hot air stream into it.

**DETERMINATION OF LEAD.** Dissolve the residue in the flask in 150 to 200 ml. of distilled water, add 2 or 3 drops of methyl red indicator, and exactly neutralize the solution with 0.0624*N* sodium hydroxide to the alkaline (yellow) end point of the indicator. Usually there will be a sufficient concentration of chlorides as a result of the preceding operations to suppress the hydrolysis of the lead. A deficiency of chlorides will render the neutral point indefinite, in which case 5 to 10 grams of sodium chloride should be added. At the neutral point one drop of 0.0624*N* acid should suffice to revert the indicator color from a canary yellow to a definite pink. Occasionally the methyl red indicator will show a fading tendency, owing to remaining traces of oxidizing substances. This fading tendency is readily overcome by the addition of a few milliliters of 0.1*N* sodium thiosulfate solution.

Where the quantity of lead present is approximately known, the titration of the lead is carried out as follows: To the neutralized solution add a 2- to 3-ml. excess of the 8-hydroxyquinoline reagent and a similar excess of the standard sodium hydroxide solution. Stopper the flask and shake vigorously for a few seconds to break up the precipitate and liberate any occluded substances. Add sufficient phenol red indicator (about 3 ml.) to produce a definite pink color and back-titrate the excess alkali with standard hydrochloric acid. The hydrochloric acid should be added dropwise toward the end of the titration and the end point taken on the yellow (acid) side of the indicator change. Agitate the flask when observing the end point and ignore any pink fluorescence which may appear after settling of the precipitate. It is advisable to redetermine the end point by adding a further excess of alkali and repeating the back-titration with acid. Make certain that there is a 2- to 3-ml. excess of the 8-hydroxyquinoline reagent over the net volume of alkali consumed.

When the approximate quantity of lead is not previously known, the titration must be carried out stepwise in order to secure the correct excess (2 to 3 ml.) of the 8-hydroxyquinoline reagent. Add about 3 ml. of phenol red indicator to the solution which has been previously neutralized to methyl red. Now add the 8-hydroxyquinoline in 3-ml. increments and after each addition add an equal volume of 0.0624*N* sodium hydroxide. An excess of the 8-hydroxyquinoline reagent is indicated when the addition of the alkali increment renders the solution alkaline (pink) to the phenol red indicator. At this point stopper the flask, shake vigorously for a few seconds, and back-titrate the excess alkali with standard acid to determine the approximate consumption of alkali. Adjust the volume of 8-hydroxyquinoline added, so that there is an excess of 2 to 3 ml. over the net volume of alkali consumed (volume of standard alkali minus volume of standard acid). A larger excess of 8-hydroxyquinoline should be avoided, as this reagent exhibits a slight buffering effect which interferes with the end-point determination. Redetermine the

end point after the addition of a further 2 to 3 ml. of standard alkali by back-titration with standard acid as already described.

Two equivalents of titratable acid are formed for each mole of lead. The solutions should be standardized against a known quantity of lead, using the same titration procedure as in the analysis. Pure test lead or lead nitrate may be used as a standard.

The result expressed in milliliters of tetraethyllead per gallon of gasoline is obtained by multiplying the PbO equivalent (expressed in grams) of the net volume of sodium hydroxide consumed by 33.24.

**USE OF POTASSIUM CHLORATE.** In order to determine the explosion hazard attending the use of potassium chlorate-nitric acid mixtures for the oxidation of organic residues, the effects of various conditions were investigated. It was found that a mild explosion would sometimes occur in the vapor if the liquid was not kept actively boiling during the oxidation process. In every case the explosion was preceded by a dense accumulation of greenish yellow vapors (probably a mixture of chlorine and organic vapors). The explosion hazard appeared to be completely eliminated by maintaining the solution in an actively boiling condition during the oxidation process, in which case the accumulation of greenish yellow fumes was prevented. By following this procedure more than 3000 samples have been analyzed without an explosion. In spite of this record it is suggested that protective mask be worn by the operator during the oxidation.

Table II. Comparison of A.S.T.M. and Proposed Methods

Samples	Tetraethyllead Content	
	A.S.T.M. method	Proposed method Ml./gallon
Competitive Q gasolines		
Brand I	0.26	0.26
Brand II	0.06	0.07
Brand III	1.26	1.25
Brand IV	0.37	0.38
Brand V	0.53	0.53
Competitive Ethyl gasolines		
Brand I	1.35	1.35
Brand II	1.78	1.80
Brand III	1.92	1.93, 1.94, 1.93
Brand IV	1.87	1.87
Brand V	1.58	1.57
Brand VI	2.01	2.01
Brand VII	1.06	1.08
Aviation gasolines		
Brand I	3.04	3.04
Brand II	3.05	3.06
Brand III	2.97, 2.97, 2.97	3.00, 3.00, 2.99

#### ACCURACY OF THE METHOD

A series of synthetic blends of tetraethyllead in cracked and straight-run gasolines was prepared to check the accuracy of the method. Blends were also prepared with the addition of isopentane and alcohol and analyzed by the described method. The results of these experiments are shown in Table I.

A comparison of results obtained by the proposed method and by the A.S.T.M. method (1) is shown in Table II.

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#### American Society for Testing Materials Meeting

The 1944 Spring Meeting and Committee Week of A.S.T.M. is to be held in Cincinnati, Ohio, at the Netherland Plaza from February 28 to March 3. The 47th Annual Meeting will be held in New York, N. Y., at the Waldorf-Astoria June 26 to 29, 1944.



# Specific Gravity of Butadiene

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The specific gravities ( $t/60^\circ \text{ F.}$ ) of 1,3-butadiene of 99.6 mole per cent purity were determined experimentally for the temperature range of  $-17.78^\circ$  to  $60^\circ \text{ C.}$  ( $0^\circ$  to  $140^\circ \text{ F.}$ ), by means of a specially constructed steel pycnometer of approximately 4500-cc. capacity and capable of withstanding the resultant vapor pressures without undergoing a permanent change in volume. The experimentally determined specific gravities were smoothed graphically and the experimental and smoothed values were compared to show the magnitude of the probable error in the smoothed data. The densities reported here were compared with densities found in the literature, and it was concluded that the final interpolated values determined in this work were probably correct to  $\pm 0.00015$ . The best value for the specific gravity ( $60^\circ/60^\circ \text{ F.}$ ) of pure 1,3-butadiene was estimated to be  $0.6274 \pm 0.00015$ .

THE current interest in 1,3-butadiene as a synthetic rubber raw material has created a need for more accurate and more complete information on the physical properties of this hydrocarbon.

Landolt and Börnstein (2) give a table of liquid densities for 1,3-butadiene for the temperature range  $-20^\circ$  to  $20^\circ \text{ C.}$  ( $-4^\circ$  to  $68^\circ \text{ F.}$ ), Prevost (4) has reported a value at  $6.22^\circ \text{ C.}$  ( $21.2^\circ \text{ F.}$ ), and Doss (1) lists a value at  $68^\circ \text{ F.}$  Because these data cover a range of temperature too small to meet most requirements, it seemed advisable to check previous values and extend the temperature range. This paper reports experimentally determined liquid specific gravities for 1,3-butadiene for the temperature range  $-17.78^\circ$  to  $20^\circ \text{ C.}$  ( $0^\circ$  to  $140^\circ \text{ F.}$ ).

## METHOD

The procedure consisted essentially of comparing the determined weights of known volumes of butadiene under a number of temperature conditions and under pressures approximately equal to the vapor pressures with the weights of identical volumes of water at  $15.56^\circ \text{ C.}$  ( $60^\circ \text{ F.}$ ) and at atmospheric pressure. This procedure has been employed previously by the writers to arrive at similar data for propane, iso- and *n*-butane, the various butylenes, and a number of commercial products falling in the classification of liquefied petroleum gases (3).

## COMPOSITION OF BUTADIENE USED

The 1,3-butadiene used in the investigation was obtained from the by-product butadiene plant of the Phillips Petroleum Company and was representative of the commercial product of the plant at the time of sampling. The sample was inhibited against oxidation with 0.02 weight per cent of phenyl-beta-naphthylamine. No solvent for the inhibitor was used. The quantity of added inhibitor was calculated to increase the specific gravity by no more than 0.00005 and its presence in the sample could, therefore, be ignored.

The composition of the sample was ascertained by two different analytical techniques, both based upon the well-known chemical reaction between maleic anhydride and 1,3-butadiene. Analyses by the two techniques gave a purity of 99.6 mole per cent. The impurities present were believed to consist of 1-butene and the high- and low-boiling 2-butenes.

## APPARATUS

The apparatus consisted of two steel pycnometers of approximately 4500-cc. capacity fitted with expansion chambers to facilitate measurements at temperatures below room temperature. A constant-temperature bath, a centrifugal pump for stirring the bath liquid, a torsion balance with calibrated weights, and a calibrated mercury-in-glass thermometer were also provided.

The details of one of the pycnometer units are shown in Figure 1, where *A* is the pycnometer and *B* is the expansion chamber. *C* is a high-pressure stainless steel needle valve of such construction that when fully opened the pressure of the material in the chambers is held by a metal-to-metal seat instead of by valve packing, thus reducing the chance for errors due to leakage. Valve *D* is a brass body steel needle valve. Both pycnometer units were tested before use with hydrogen gas at 27-kg. (600 pounds) pressure to assure absolute freedom from leaks. The two units were used simultaneously for check determinations.

The thermometer used for the measurement of bath temperatures was graduated in  $0.2^\circ \text{ F.}$  divisions. It was checked before use against a Bureau of Standards calibrated mercury-in-glass thermometer.

The torsion balance was checked before use for accuracy, sensitivity, stability, and equality of length of balance arms. It was tested during use for reproducibility of weights by weighing an iron weight of about 9 kg. (20 pounds) at various times during the day and on successive days. These tests indicated that a weight in the desired range—i.e., 7.7 to 8.6 kg. (17 to 19 pounds)—could be reproduced to 68 mg. ( $\pm 0.0015$  pound). The set of brass weights used were calibrated to 9 mg. ( $\pm 0.0002$  pound).

**CALIBRATION OF PYCNOMETERS.** After being carefully cleaned and dried, both internally and externally, the two pycnometer units were evacuated and the tare weights determined and checked by repeated weighings to the nearest 22 mg. (0.0005 pound). The volumes of the pycnometer chambers were then ascertained for temperatures of  $4.44^\circ$ ,  $15.56^\circ$ ,  $26.67^\circ$ ,  $37.78^\circ$ ,  $48.89^\circ$  and  $60^\circ \text{ C.}$  ( $40^\circ$ ,  $60^\circ$ ,  $80^\circ$ ,  $100^\circ$ ,  $120^\circ$ , and  $140^\circ \text{ F.}$ ), and with no internal pressure on the chambers, by weighing the water-filled chambers at the various temperatures and then making corrections for the changing density of water. Freshly boiled distilled water was used. The effect of internal pressure on pycnometer chamber volumes was ascertained for a temperature condition of  $15.56^\circ \text{ C.}$  ( $60^\circ \text{ F.}$ ) and pressures of 0, 14, and 28 kg. per sq. cm. (0, 200, and 400 pounds per square inch) gage, respectively. The final results of the calibrations expressed in terms of volume for various conditions of temperature and internal pressure, were plotted to arrive at a smooth relationship for use in the subsequent experiments. It is believed that the finally assigned volumes for the various conditions were known to  $\pm 0.2 \text{ cc.}$  for the entire temperature range.

## MEASUREMENT OF DENSITIES

The pycnometer units were evacuated to an absolute pressure of less than 1 mm. of mercury. Sufficient butadiene was then charged into the units to fill *A* completely and *B* to half its capacity. Valve *D* was closed and *C* was left open. The units were then placed in a constant-temperature bath in such a manner that cell *A* was totally immersed in the bath liquid, but with no part of cell *B* immersed. Heat was applied externally to *B* by means of an electrically heated removable jacket to maintain its temperature,  $6^\circ$  to  $9^\circ \text{ C.}$  ( $10^\circ$  to  $15^\circ \text{ F.}$ ) higher than the bath temperature. The temperature of *B* was measured by a thermocouple on the outside surface of the cell at a point below the liquid level in the cell. In a preliminary series of observations it was determined that approximately one hour was required to bring the temperature of the pycnometer and its charge of butadiene to the bath temperature. The pycnometers were consequently held in the constant temperature bath for 1.5 hours before being

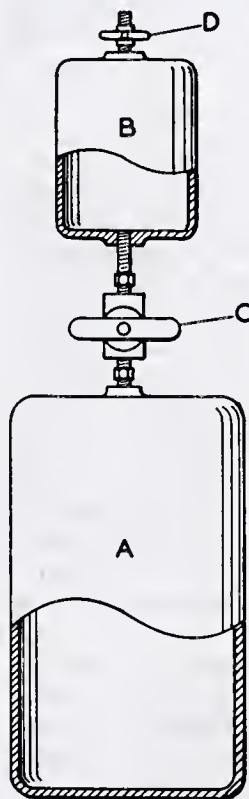


Figure 1. Metal Pycnometer Unit



Table I. Summary of Experimentally Determined Specific Gravities of 1,3-Butadiene

This Work (99.6 Mole Per Cent 1,3-Butadiene)			
Temperature ° F.	Pycnometer No.	Specific gravity ( <i>t</i> /60° F.) <sup>a</sup>	Literature, Specific Gravity ( <i>t</i> /60° F.) <sup>a</sup>
0	1	0.66671	0.6659 (2) <sup>b</sup>
	1	0.66671	
	2	0.66675	
	2	0.66675	
20	1	0.65414	0.6640 (2) <sup>b</sup>
	2	0.65410	
21.2			0.6493 (4)
40	1	0.64097	0.6409 (2) <sup>b</sup>
	1	0.64097	
	2	0.64070	
	2	0.64065	
60	1	0.62732	0.6273 (2) <sup>b</sup>
	2	0.62725	
68			0.6213 (1)
80	1	0.61361	....
	2	0.61371	
100	1	0.59933	....
	1	0.59944	
	1	0.59944	
	2	0.59932	
	2	0.59937	
	2	0.59947	
120	1	0.58445	....
	2	0.58422	
140	1	0.56920	....
	1	0.56890	
	2	0.56850	
	2	0.56870	

<sup>a</sup> Specific gravity at temperature *t* with reference to water at 60° F.<sup>b</sup> Interpolated values.

Table II. Smoothed Specific Gravity Values for Commercially Pure 1,3-Butadiene

(99.6 mole per cent 1,3-butadiene)			
Temperature ° F.	Specific Gravity ( <i>t</i> /60° F.) <sup>a</sup>	Temperature ° F.	Specific Gravity ( <i>t</i> /60° F.) <sup>a</sup>
0	0.6668	72	0.6191
2	0.6655	74	0.6177
4	0.6643	76	0.6163
6	0.6631	78	0.6149
8	0.6618	80	0.6135
10	0.6606	82	0.6121
12	0.6593	84	0.6106
14	0.6580	86	0.6091
16	0.6567	88	0.6077
18	0.6554	90	0.6063
20	0.6541	92	0.6049
22	0.6529	94	0.6035
24	0.6515	96	0.6021
26	0.6502	98	0.6007
28	0.6489	100	0.5993
30	0.6476	102	0.5978
32	0.6463	104	0.5963
34	0.6449	106	0.5949
36	0.6436	108	0.5934
38	0.6423	110	0.5919
40	0.6409	112	0.5904
42	0.6396	114	0.5889
44	0.6382	116	0.5874
46	0.6369	118	0.5859
48	0.6356	120	0.5844
50	0.6342	122	0.5828
52	0.6328	124	0.5813
54	0.6314	126	0.5798
56	0.6300	128	0.5783
58	0.6286	130	0.5767
60	0.6273 <sup>b</sup>	132	0.5751
62	0.6259	134	0.5736
64	0.6245	136	0.5720
66	0.6232	138	0.5704
68	0.6218	140	0.5689
70	0.6205		

<sup>a</sup> Specific gravity at temperature *t* with reference to water at 60° F.<sup>b</sup> Probable specific gravity (60°/60° F.) for pure 1,3-butadiene, arrived at by applying corrections for assumed impurities, was estimated to be 0.6274 ± 0.00015.

removed for weighing. During that time, the bath temperature was held constant to 0.06° C. (±0.1° F.). Just before removing a pycnometer from the bath, *C* was closed. Immediately upon removal, the outside surface was dried and *B* was evacuated. *D* was then closed, *C* was opened, and the unit was weighed. In those instances where the bath temperature was below the dew point temperature of the room air, the temperature of the pycnometer was raised to above room temperatures to avoid errors in weighing occasioned by condensation of moisture on the surface. In such cases *B* served as a receiver for the liquid butadiene displaced from *A*.

## RESULTS

The specific gravities of the butadiene were subsequently arrived at by dividing the determined weights of the butadiene contained in *A* at the various temperatures by the weight of the same volume of water when at 60° F. and atmospheric pressure. The value for the density of water at 60° F. used in these calculations was taken as 0.999017 gram per cc. All experimental results are presented in Table I together with comparable data from Doss (1), Landolt and Börnstein (2), and Prevost (4).

An analysis of the method used showed that errors in determined gravities traceable to air buoyancy effects were of small magnitude. The buoyancy correction, calculated to be +0.00003, was not applied since it was too small in comparison with the experimental error to be significant. Within the experimental error it has been concluded that the determined specific gravity values can be accepted as equivalent to those taken in a vacuum.

The specific gravities presented in Table I were plotted against temperature, and, from a smooth curve drawn through the points, the specific gravity values for the various intermediate temperatures were determined. These smoothed specific gravity values are presented in Table II. Of the twenty-two different specific gravity measurements made at temperatures of 48.89° C. (120° F.) and below, only those made at 4.44° and 26.67° C. (40°

and 80° F.) in pycnometer unit 2 differed from the smoothed value by more than the predicted probable amount of 0.00018.

It was believed that the 0.4 mole per cent of impurities present in the sample consisted of 1-butene and the high- and low-boiling 2-butenes. By making certain assumptions, it was possible to predict the specific gravity at 60° F. of pure 1,3-butadiene. Thus, if it were assumed that the three probable impurities were present in substantially equal proportions, the computed value of pure 1,3-butadiene would be higher than the determined specific gravity of the test material by 0.00007. On adding the buoyancy correction of 0.00003 and subtracting the correction of 0.00005 for the amount of inhibitor present, the final value rounded off to 0.6274 was obtained for the specific gravity (60°/60° F.) of pure 1,3-butadiene. This was considered to be the most nearly correct value for pure 1,3-butadiene at 60° F.

## ACKNOWLEDGMENT

The writers wish to acknowledge the helpful suggestions made by various members of the Phillips Petroleum Company Research Department in the development of the experimental method and, in particular, the assistance rendered by L. R. Fruit on all experimental measurements. Acknowledgment is also made both to Phillips Petroleum Company and to the B. F. Goodrich Company for the use of certain analytical techniques.

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# Physical Methods of Analysis of Synthetic and Natural Rubber

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In a compounded rubber stock, the ratio of natural to synthetic rubber can be estimated approximately from a knowledge of the phosphorus content of the rubber hydrocarbon. A considerably more exact analysis can be carried out by means of infrared spectroscopic methods, which permit a determination of the type as well as the amount of rubber present. Complete details and comparative results of these two methods of analysis are given, as well as a simple procedure for separating the rubber hydrocarbon of a rubber stock from the carbon black and other compounding ingredients.

REPORTS have been made that more than 3000 kinds of rubberlike synthetics have already been prepared. Obviously, not all are of practical importance, nor can they legitimately be referred to as "synthetic rubbers", but a certain select group of these, as well as still other new synthetics, will survive and prove meritorious. Uses will be found for these new synthetic rubbers both alone and when blended with other synthetics or with natural rubber. The rubber chemists of the future will therefore be faced with the problem of analyzing such mixtures. Because the components of these mixtures are often complex and their degradation products are not well known, it will be difficult, by conventional analytical methods, to establish their identities or the proportions in which they are present.

In anticipation of these difficulties, it was deemed advisable to study the applicability of various physical tools to this and other problems of the rubber industry. In a previous publication (1) the authors called attention to the fact that infrared spectroscopy could be of value in differentiating between rubbers and in analyzing rubber mixtures, and pointed out that the infrared absorption spectra of the various rubbers are unique and offer one means of attacking the analytical problem outlined. However, in this preliminary paper the analytical possibilities were merely noted without any attempt to reduce them to practice.

Shortly after the completion of this preliminary study, the authors were called upon by the War Production Board to analyze a series of captured German tires and inner tubes for the Army Ordnance Department, and to determine, if possible, the amounts and types of synthetic rubbers which had been blended with natural rubber in the manufacture of these tires. As a result of this investigation, two satisfactory methods of analysis were developed.

The first, the determination of the phosphorus content, furnishes a simple method for measuring the relative amounts of natural and synthetic rubbers present in an unknown; the second, the application of infrared spectroscopy, permits determination of both the type of each rubber and

the amount present. In conjunction with the latter analysis, a method was devised for separating from compounded rubber products a sample of rubber hydrocarbon free of pigment and filler. The details of the preparation and analysis of samples are given, together with a typical set of data obtained in connection with the tire and tube studies.

Although these methods may not necessarily be successful in solving every type of rubber mixture analysis encountered, their value in this particular problem warrants careful consideration in connection with similar problems which may arise in the future.

## DETERMINATION OF RATIO OF NATURAL TO SYNTHETIC RUBBER BY PHOSPHORUS CONTENT

The metabolic processes of plants bring about, within the various parts of the plant, the deposition of a great many of the metals commonly found in the soil. In contrast with this, the metals found in any synthetic product are limited to those purposely added and those accidentally introduced by contact with the pieces of processing equipment.

In order to determine whether this difference might prove to be valuable for analytical purposes, samples of natural and synthetic rubbers were subjected to an ultraviolet spectrochemical analysis, using a large Hilger E-1 spectrograph. Table I shows the results of such a comparative emission analysis. It may be seen at once that, whereas the synthetics contain little or no phosphorus, this element is present in the natural rubbers in readily detectable quantities. This clue was followed up in great detail and the exact values for the phosphorus contents were determined through the use of another spectrochemical

Table I. Ultraviolet Spectrochemical Analysis of the Metal Content of Rubber Hydrocarbons

	Natural Rubber					Synthetic Rubber		
	Smoked sheet	Crepe rubber	Tread stock	Carcass stock	Re-claimed tires	Domestic Buna S	German Buna S	Buna S tread stock
	(0.27)	(0.17)	(3.6)	Per Cent Ash (32.5)	(23.2)	(1.49)	(2.11)	(6.3)
Aluminum	2-	2-	2+	2-	3-	2-	2+	2
Antimony	0	1	0	0	2	0	0	0
Barium	1+	1+	1+	1+	3-	2-	2-	1+
Boron	1+	1+	2-	2-	1	1+	1+	2-
Cadmium	0	0	1	1+	2-	0	1-	1
Calcium	2	2	2+	2-	3-	2+	2+	3-
Chromium	1	1	1+	0	2-	1+	1+	2-
Copper	2-	2-	1+	1+	2-	2-	2-	1+
Iron	2-	2-	2	2	2	2	2	2
Lead	1	1	1+	2-	2	1+	1+	1+
Magnesium	2+	2+	3-	2+	3-	2+	3-	3-
Manganese	1	1	1+	1	1+	2-	1+	1
Molybdenum	0	0	0	0	2-	0	0	0
Nickel	1	1	1+	0	1+	1+	1+	2-
Phosphorus	2+	2	2+	2	2	0	1+	0
Potassium	2	2	0	0	2-	2-	0	1+
Silicon	2	2	2+	2	2+	2	2+	3-
Sodium	2	2	2	2	3-	2+	2	2+
Strontium	1+	1+	1	0	2	1+	1+	1
Tin	1	1	1	1	1	1+	1	1
Titanium	1+	1	1+	1	2	1	2	1
Vanadium	0	0	0	0	0	0	2-	0
Zinc	2-	2-	3-	3	2+	2	2-	3-

Ranges for qualitative estimates:

3 = 100 to 1.0%

3- = 10 to 0.10%

2+ = 1.0 to 0.01%

2 = 0.1 to 0.001%

2- = 100 to 1.0 p.p.m.

1+ = 10 to 0.10 p.p.m.

1 = 1.0 to 0.01 p.p.m.

1- = less than 0.1 p.p.m.

0 = metal not detected



tool, the visible light spectrophotometer. The details of the exact procedure followed are given below.

Table II gives the phosphorus contents of a variety of natural and synthetic rubbers. These values show a considerable variation for natural rubbers of different origins, but all may be characterized by high phosphorus (average 400 p.p.m.) when compared with typical synthetics (average 20 p.p.m.). Thus, an exact phosphorus determination should make possible a determination of the ratio of natural to synthetic rubber in an unknown sample.

Table II. Phosphorus Content of Various Rubbers

	P.p.m.		P.p.m.
Natural rubber		Synthetic rubber	
Smoked sheet A	690	Buna S (American)	15
Smoked sheet B	380	Buna A (German)	30
Smoked sheet C	320	Cotton tire cord	210
Smoked sheet D	500	Viscose tire cord	10
Smoked sheet E	400		
Crepe	490		
Pale crepe	350		
Reclaimed tube	390		
Guayule	200		

Figure 1 was plotted in order to show that the phosphorus could be determined accurately enough to allow the content of this element to be used as a yardstick and as a direct measure of the amount of natural rubber present. (If the origin of the natural rubber is unknown, the slope of this curve is not determinable, and the method is only roughly quantitative. On the other hand, if a sample of the natural rubber used in compounding is available for a phosphorus test, a much higher degree of accuracy may be expected.) In spite of the variation of the phosphorus content of natural rubbers of different origin, a reasonable first guess at a mixture of unknown constitution may be based upon the following generalization:

High phosphorus (300 to 450 p.p.m.)	= natural
Medium phosphorus (100 to 250 p.p.m.)	= natural + synthetic
Low phosphorus (0 to 50 p.p.m.)	= all synthetic

As will be seen from Table IV, the above method provides a very simple approximate analysis. The agreement between this and the more exact infrared method is indeed surprising. However, the method is always open to the uncertainty that phosphorus may have been introduced in processing and not been eliminated in the sampling procedure.

Although any sensitive method for determining the phosphorus will be satisfactory, the spectrochemical procedure detailed below was found to be most suitable. This colorimetric method is based upon many literature references of which Zinzadze (10) and Goodloe (6) are typical. An intense blue color is produced in solutions containing phosphorus by reduction of phosphomolybdate, the intensity of the color being directly proportional to the phosphorus content of the solutions. In this laboratory, the color values or per cent transmissions were measured on a General Electric recording spectrophotometer. The blue color which develops has a rather broad spectral width which can be measured satisfactorily by means of any ordinary comparison colorimeter, although the increased precision of the more accurate photoelectric instruments is to be preferred. Figure 2 shows a series of transmission curves for standard phosphorus solutions.

**PREPARATION OF SAMPLE.** All glassware and reagents must be free of phosphorus and a suitable blank or control must be carried through the complete procedure.

Other possible sources of extraneous phosphorus are the cord and plasticizer used in fabrication. [It is interesting in this connection to note (Table II) that cotton, or natural, fibers are high in phosphorus, whereas synthetic fibers, rayons, etc., have a low content, thus enabling a distinction to be made between these two types of fibers. Undoubtedly other cases of natural

versus synthetic materials may arise in which this general method might be of help.] Therefore, it is advisable that all cords, if present, be removed by the following procedure: A section of the rubber (tire) is shredded in a two-roll mill, then mixed with water in a Waring Blendor. After 5 to 10 minutes' stirring, the cord can be partially separated by decantation. The remaining cord is digested at about 4° C. for 18 hours with an excess of cuprammonium solution [see Clibbens and Gaeke (5) and the committee of the AMERICAN CHEMICAL SOCIETY (4) for preparation of this solution]. The material is washed free of cuprammonium with water and then dried. If the sample is prepared rubber stock, all phosphate-containing plasticizers must be removed by refluxing the shredded rubber for 8 hours in a Soxhlet extractor with a solvent composed of 68% chloroform and 32% acetone.

For maximum photometric accuracy, a weight of rubber should be taken such that a phosphorus content up to 0.080 mg. is contained. With synthetic rubbers, a 1-gram sample is suitable, whereas, with natural rubber, approximately 0.1 gram is used. In order to ensure a representative sample, a larger weight is taken and subjected to the preliminary preparation. The color value of a 1-gram equivalent is then measured to furnish a rough idea of the phosphorus content. With this as a basis, a weight of sample is chosen according to the above criterion and is measured accurately.

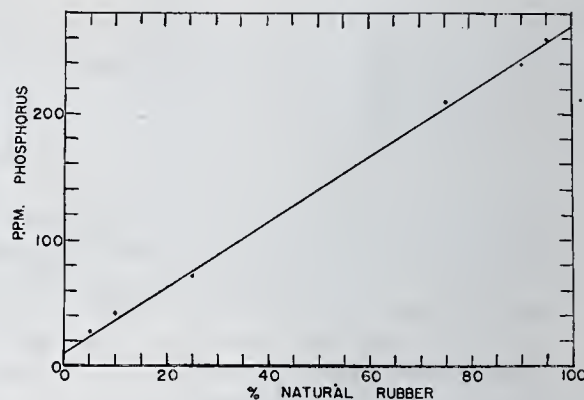


Figure 1. Phosphorus in Carcass Stock Compounded with Natural Rubber and Buna S

Formula of Stock

Rubber hydrocarbon	100
Zinc oxide	50
Stearic acid	1
Pine tar	1.5
Agerite resin	1.5
Sulfur	2.0
Altax	1.25

Preferably, the phosphorus values should be calculated to the weight of rubber hydrocarbon, the carbon, zinc oxide fillers, etc. having been removed previously. This was not always done in the authors' work, as the carbon analysis was done elsewhere; hence, the phosphorus content of the samples reported here was based on the entire rubber sample.

**ASHING OF SAMPLE.** The weighed sample of extracted rubber is ashed in a suitable crucible in a controlled furnace at a temperature not higher than 600° C. until free of organic material. This must be done slowly enough to prevent the material from kindling. It was not found necessary to add a phosphorus fixative in the case of rubber materials, as no increased phosphorus values were obtained by adding zinc oxide to prevent the volatilization of phosphorus. After cooling, the residue is dissolved by boiling in dilute sulfuric acid in a quantity just sufficient to dissolve the soluble materials. Excess acid is to be avoided, since the final acidity is very important, but an excess of acid may be added and later neutralized. A slight turbidity which may be removed by filtering remains in some cases as a result of the presence of a siliceous material. The clear solution of the ash is transferred to a 50-ml. Pyrex volumetric flask and the volume is adjusted to about 40 ml.

**DEVELOPMENT OF COLOR.** Although many procedures are described in the literature, the following modification was used here.

**Standard Solutions.** Solution I, ammonium molybdate, 5.4 grams  $[(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}]$ , dissolved by warming with water and made to 100 ml.

Solution II, 10N sulfuric acid (282 ml. of concentrated sulfuric acid diluted to 1000 ml.), checked by titration.



Solution III, stannous chloride. A stock solution of stannous chloride dehydrate is 40 grams dissolved in concentrated hydrochloric acid (density 1.18) and made up to 100 ml. (stable for months). For use, it is diluted 200 times with distilled water (stable for one day).

**Analytical Procedure.** To the solution prepared according to instructions above, 2.5 ml. of Solution I and 5 ml. of Solution II are added and mixed thoroughly, and 2 ml. of Solution III are added with constant swirling. The solution is made to volume (50 ml.) and mixed thoroughly. (The final acidity of the 50-ml. sample should be 1 N sulfuric acid. If excess acid is used in dissolving the ash, it may be neutralized at that point or the amount of Solution II may be reduced to give the final acidity stipulated.) A blank is carried through the same procedure to check for the presence of phosphorus in the reagents.

**DETERMINATION OF PHOSPHORUS CONTENT.** The per cent transmission of the solution in a 1-cm. thick cell at 700 mμ is measured exactly 20 minutes after adding Solution III. The amount of phosphorus in an unknown may be determined from a calibration curve which shows the per cent transmission plotted as ordinates versus the phosphorus content of known standard solutions as abscissas. The calibration curve may be prepared from a series of transmission curves such as those of Figure 2.

**NOTES ON PHOSPHORUS ANALYSIS.** When a molybdate is added to a solution containing orthophosphate according to the method described above, an insoluble phosphomolybdate is formed. However, because of the high dilution, a precipitate is not apparent. The addition of a reducing agent (stannous chloride) causes a reduction of the phosphomolybdate and gives an intense blue color. Under the proper conditions, the molybdenum reagent necessary as an excess is not reduced.

The factors which affect the color are:

1. The acid concentration is very important. In the presence of too much acid, a light color is produced. Conversely, if too little acid is used, the molybdate reagent itself will be reduced, causing dark colors. In the method used, a 20% decrease in acidity results in a 10% increase in color intensity, while a 10% increase in acidity results in a 10% decrease in color intensity. Soluble silica also produces a blue color with the above reagent if the silica concentration is high or the acidity is insufficient to suppress the ionization of the silicic acid. The acidity chosen as optimum in this investigation (1N sulfuric acid) is such that silica up to 2000 p.p.m. does not interfere.

2. The molybdate concentration is the next most critical factor. An increased molybdate concentration results in a higher sensitivity to phosphorus, but also an increased blank. A high blank reading is not desirable when low concentrations of phosphorus are present. A 50% increase in the molybdate concentration, as used above, results in approximately a 15% increase in the color intensity. A 20% decrease in molybdate concentration results in approximately a 10% decrease in color intensity.

3. The stannous chloride concentration is not very critical. A ±50% change in the amount of stannous chloride influences the phosphorus result not more than ±5%.

4. Under the given conditions, the color increases for 5 to 10 minutes after the stannous chloride is added and bleaches slowly thereafter. It is recommended therefore that the color be measured 20 minutes after addition of the stannous chloride.

5. The following sources of interference have been considered, and methods of reduction or elimination are recommended where necessary:

Ferric ion up to 6 p.p.m. does no harm. Fifteen parts per million slightly inhibit color development, while larger amounts of ferric ion cause very rapid fading of the color. Ferrous ion causes no harm. A Jones reductor with metallic cadmium gives best results for prevention of interference from ferric ion (?).

The presence of more than 20 p.p.m. of titanium causes interference by retarding the rate of color development.

Arsenates give the same color as phosphates and the intensities are inversely proportional to the molecular weights. Reduction with sodium bisulfite eliminates the influence of arsenates (20 mg. of arsenic pentoxide per 50 ml. may be taken care of by reduction to arsenic trioxide).

Nitrates up to 100 p.p.m. have no effect; 200 p.p.m. reduce the color about 10%.

Sulfates in large amounts interfere, presumably by depressing the ionization of the sulfuric acid.

Tartaric and citric acids interfere above 20 p.p.m., with inhibition of maximum color. They may be removed by oxidation with permanganate.

Aluminum and manganese in reasonable amounts do not interfere.

Calcium and magnesium up to 1000 p.p.m. have no effect.

Nickel up to 1000 times the phosphorus content does not interfere except for its own color.

Trichloroacetic acid begins to interfere with the maximum color development at concentrations above 4% in the final mixture. Acetic acid shows practically no effect.

Hydrochloric acid has a tendency to lessen color stability and retards or inhibits maximum coloration.

DETERMINATION OF TYPES AND AMOUNTS OF RUBBERS BY INFRARED SPECTROSCOPY

Recent publications (1, 2, 3, 8) have described in great detail the methods and applications of infrared spectroscopy to the identification and analysis of many types of organic materials. It is sufficient here to point out the two salient characteristics of infrared absorption in order that the basis for analysis can be understood.

In the first place, the infrared absorption spectrum (a plot of per cent transmission as ordinate versus frequency in cm.<sup>-1</sup>) of a material is a unique characteristic of the material and cannot be duplicated by another compound. Some of the absorption bands can be ascribed to particular atomic groups within the molecule while others, characteristic of the molecule as a whole, are particularly useful for such studies as differentiating isomers. Thus it is to be expected that the phenyl group in Buna S would give rise to absorption bands which would not be present in natural rubber, while conversely, the methyl groups of natural rubber would cause a characteristic absorption which would not be observed in Buna S. Moreover, it is to be expected that there will be further bands characteristic of the molecule as a whole which will assist in the differentiation.

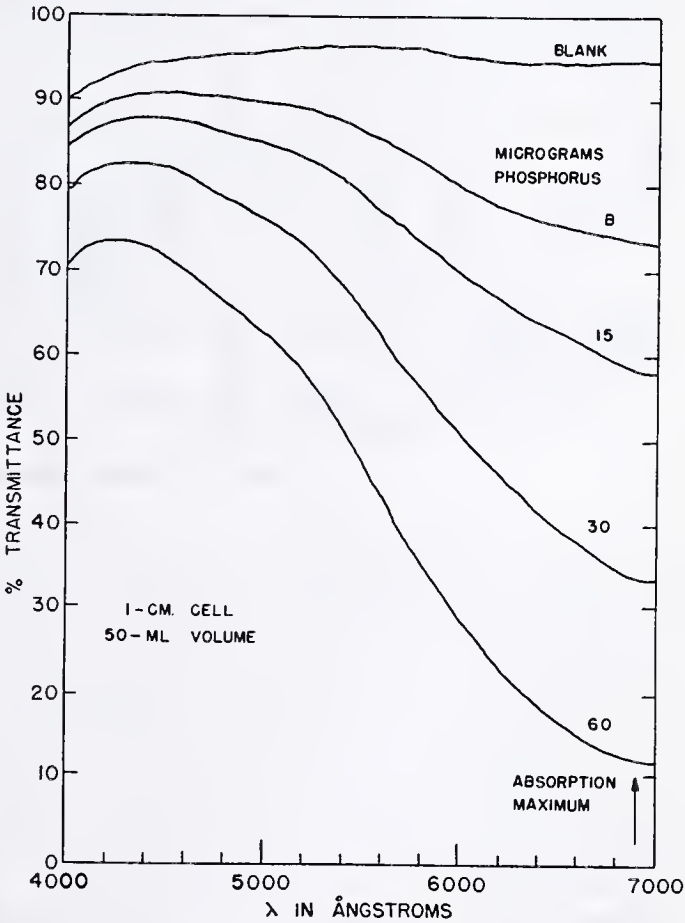


Figure 2. Spectrophotometric Determination of Phosphorus

Calibration of phosphorus as reduced phosphomolybdate  
Final solution 1N in sulfuric acid, MoO<sub>3</sub> 0.13 gram per 50 ml., SnCl<sub>2</sub>·2H<sub>2</sub>O 0.006 gram per 50 ml.

T	-log T	Blank	Net -log T	Phosphorus Micrograms	P, Microgram
%					
94.5	0.024			None	
73	0.137	0.024	0.113	8	0.0141
58	0.237	0.024	0.213	15	0.0142
34	0.469	0.024	0.445	30	0.0148
12.5	0.903	0.024	0.879	60	0.0146



In the second place, so long as no intermolecular action occurs, the spectrum of a mixture of rubbers will be simply the spectra of the pure components combined in the proportion in which the materials themselves are present. Hence, it is to be expected that an unknown mixture can be analyzed by direct measurement of the strength of absorption bands unique to each component, or by comparison of the absorption spectra of the unknown with those of a series of known prepared standards.

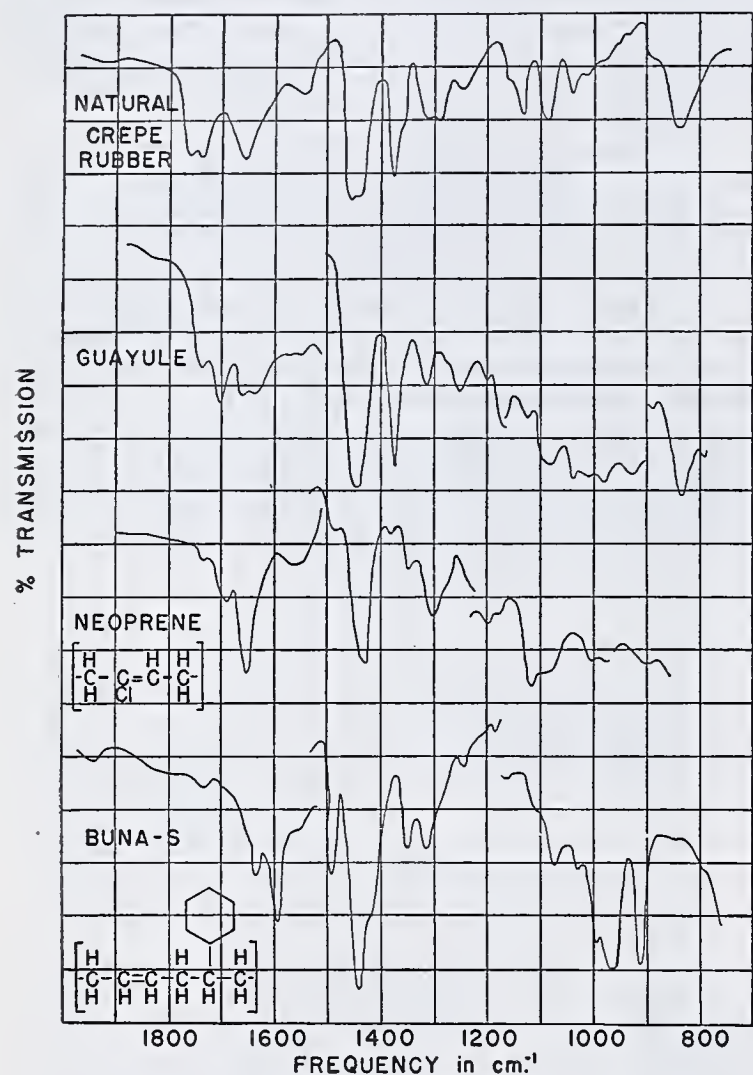
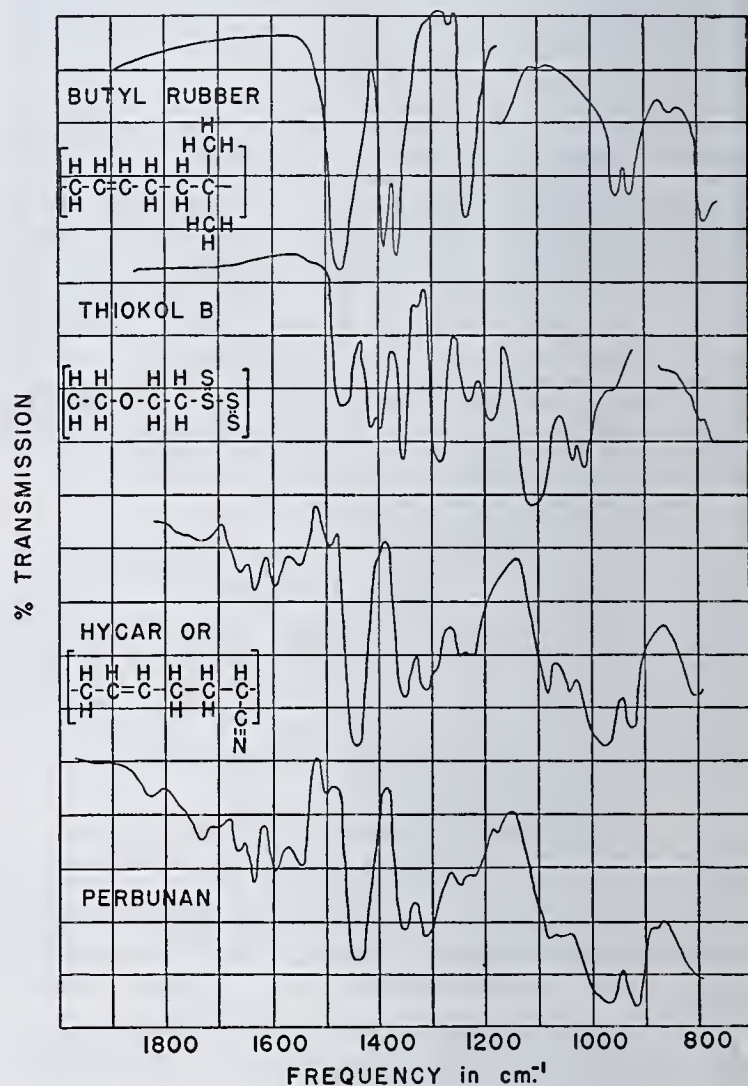


Figure 3. Infrared Absorption Spectra of Rubber Hydrocarbons

The infrared absorption spectra of various common types of rubber are shown in Figure 3 (1). The differences in these spectra readily show their applicability in identifying an unknown rubber or in estimating the content of a mixture. In the course of this investigation, only mixtures of natural rubber and Buna S were encountered. A consideration of these two spectra in Figure 3 or Figure 4 shows several major points of difference—Buna S has a strong aromatic ring frequency near  $1500\text{ cm}^{-1}$  and two strong bands at  $970\text{ cm}^{-1}$  and  $917\text{ cm}^{-1}$  which are not present in the natural rubber. Natural rubber, on the other hand, shows a methyl band at  $1380\text{ cm}^{-1}$  and a strong band at  $835\text{ cm}^{-1}$  which are not observed in Buna S. Taking advantage of these differences, each unknown sample was analyzed by comparison of its spectrum with the spectra of a series of known mixtures compounded and treated in approximately the same way as the unknown materials. The method is outlined roughly in Figure 4, showing the absorption spectra of natural rubber, 50% natural and 50% Buna S, pure Buna S, and an unknown tire tread. It can be seen from the 50-50 mixture that the unique bands mentioned above are all present with a strength proportional to the component concentration.

In the actual analysis, a great many intermediate standard spectra were obtained. An unknown was matched with one of

this series by a comparison of the absorption intensities  $1500\text{ cm}^{-1}$ ,  $917\text{ cm}^{-1}$ ,  $1380\text{ cm}^{-1}$  and  $835\text{ cm}^{-1}$ . By this method, an unknown such as that shown in Figure 4 could be estimated to be 85% natural rubber, 15% Buna S. All absorption spectra were taken with samples smeared on salt plates and a high resolution spectrometer (2) was employed. The accuracy of the method is limited to 5 to 10% because of the inability to make smear samples of



constant thickness. Greater accuracy, if desired, could be obtained by making per cent transmission measurements at the chosen frequencies according to some of the more involved analytical methods referred to above (2, 3, 8). In order to justify the careful treatment, it would be necessary to study the unknowns and standards as solutions of known concentration in a suitable solvent which would transmit infrared radiation at the analytical frequencies chosen. This more careful treatment would still require a preliminary complete absorption spectrum of each unknown, in order to furnish a qualitative analysis for the various rubber materials which are present.

**SAMPLE PREPARATION.** The methods described above are standard procedures and are given in detail in the reference. Considerable time, however, had to be devoted to finding a means of converting a piece of tire stock to a sample whose infrared absorption spectrum could be measured. All attempts to obtain the spectra of the tire stocks directly, either by microtoming thin section or by a smear from a solution of the stock materials were unsuccessful. In order to obtain a satisfactory spectrum it is necessary to extract the rubber hydrocarbon free of plasticizer, filler, etc. The method finally devised was completely satisfactory for the authors' purpose and should be of great value



any situation where it is desirable to separate filler and rubber without destruction of the rubber hydrocarbon. Therefore, this separation by means of *p*-cymene and xylene solution is completely described below.

After this separation, the rubber is present as a solution in the hydrocarbon solvents. These solvents are removed by vacuum distillation until the rubber is a gummy solid that will not flow at room temperature. This gum is then washed four or five times with hot acetone to remove the last traces of plasticizer and solvent.

In most cases the state of the rubber is such that it can easily be smeared on a rock salt plate. The desired thickness is obtained by scraping the film with a razor blade until the 1450  $\text{cm}^{-1}$  CH band shows a transmission of about 10%. If the rubber does not spread easily, it may be softened with a volatile solvent such as carbon tetrachloride. The film is then dried in a vacuum oven to remove the last traces of solvent. Finally, the absorption spectrum of the sample is obtained throughout the spectral region of interest.

DISSOLVING RUBBER HYDROCARBON AND SEPARATING IT FROM COMPOUNDING INGREDIENTS

In the case of vulcanized natural rubber compositions, various solvent methods of determining the amount of rubber hydrocarbon have been used with some success. These methods, however, are somewhat involved and time-consuming. Even the A.S.T.M. method requires that the solution stand overnight to allow the mineral fillers and pigments to settle before filtration. If the rubber composition contains highly dispersed, exceedingly fine pigments, such as carbon black, separation of the pigments by filtration or centrifuging is usually impossible.

Since many present-day rubber products, particularly tire tread compositions or compounds, contain large amounts of carbon black, none of the above-mentioned methods appeared promising for separation of the rubber from the carbon black in suitable condition for infrared analysis. Accordingly, research on this question was initiated.

Fearing that destruction or decomposition of the rubber hydrocarbons might result from the use of the conventional high-boiling solvents used in the above separation methods, a number of low-boiling solvents were tried: ethylene dichloride, toluene plus piperidine, *o*-nitroanisole, Dispersing Oil No. 10 (Barrett) plus xylene, and Circolight Process Oil (Sun Oil Co.) plus xylene. These experiments were unsuccessful.

**METHOD I.** In the next attempts, which were somewhat more successful, xylene plus a small amount of thio- $\beta$ -naphthol boiling under reflux was found to dissolve natural or synthetic tire tread stocks in 5 to 6 hours. To accomplish this, 1 gram of chloroform-acetone-extracted tread stock was heated in 100 cc. of xylene plus 0.3 gram of thio- $\beta$ -naphthol at 140° C., until solution was complete.

However, this solution did not permit easy separation of the carbon black unless a combination of centrifuging and slight alcohol precipitation was used. (By adding alcohol to the solution, a small amount of rubber is precipitated in order to drag down the carbon black during centrifuging. There is some danger that this precipitation may be selective and thus change the composition of the residual rubber.) A sufficient separation of the carbon black can be made in this manner to permit fairly satisfactory infrared analysis. In the case of carcass stocks containing only zinc oxide as a filler, the pigment was easily separated by settling or centrifuging. This method was not completely satisfactory, however, because of carbon black troubles and the difficulty of removing the peptizer without loss of the rubber itself.

The search for a better means of carbon black separation was therefore continued. The cresol method (9) was tried with some success, although there was considerable oxidation of the rubber at the high reflux and distillation temperature required. The use of *p*-cymene in place of the cresol with digestion at about 160° to 170° C. gave a satisfactory solution in about 4 hours and caused no noticeable oxidation of the rubber. On dilution with the benzene and 70° Bé. rubber solvent gasoline, the supernatant liquid

showed clearing in a few minutes and could be filtered free of the carbon black after standing 10 minutes. This method was further altered slightly by using 250 cc. of *n*-hexane instead of 300 cc. of 70° Bé. gasoline for diluting the solution. Again the carbon black separated easily. In order to determine whether or not the same dilution technique would work with xylene as the solvent, a solution of the same tread stock was made with 30 cc. of xylene plus a small amount of thio- $\beta$ -naphthol, but on diluting with benzene and hexane, the carbon black would not separate from the solution.

Further experiments showed that the combined use of *p*-cymene and xylene gave the most successful results and led to a satisfactory method.

**METHOD II** (adopted for solution of rubber and separation of pigments). Sheet the sample to a thickness of approximately 0.375 cm. (0.15 inch) on a tight cold 15 × 30 cm. (6 × 12 inch) laboratory mill.

Extract the plasticizer, etc., from 4 grams of the sheeted sample with a mixture of 32% by volume of acetone and 68% by volume of chloroform for a minimum of 7 hours, or until the extracting liquid no longer shows color. This size of sample is sufficient to permit repeat analyses.

Place 1.0 gram of the dried, extracted sample in a 400-cc. rubber extraction flask with 25 cc. of *p*-cymene and 5 cc. of xylene. Heat on a steam bath at about 70° to 80° C. for 1 hour, then on a

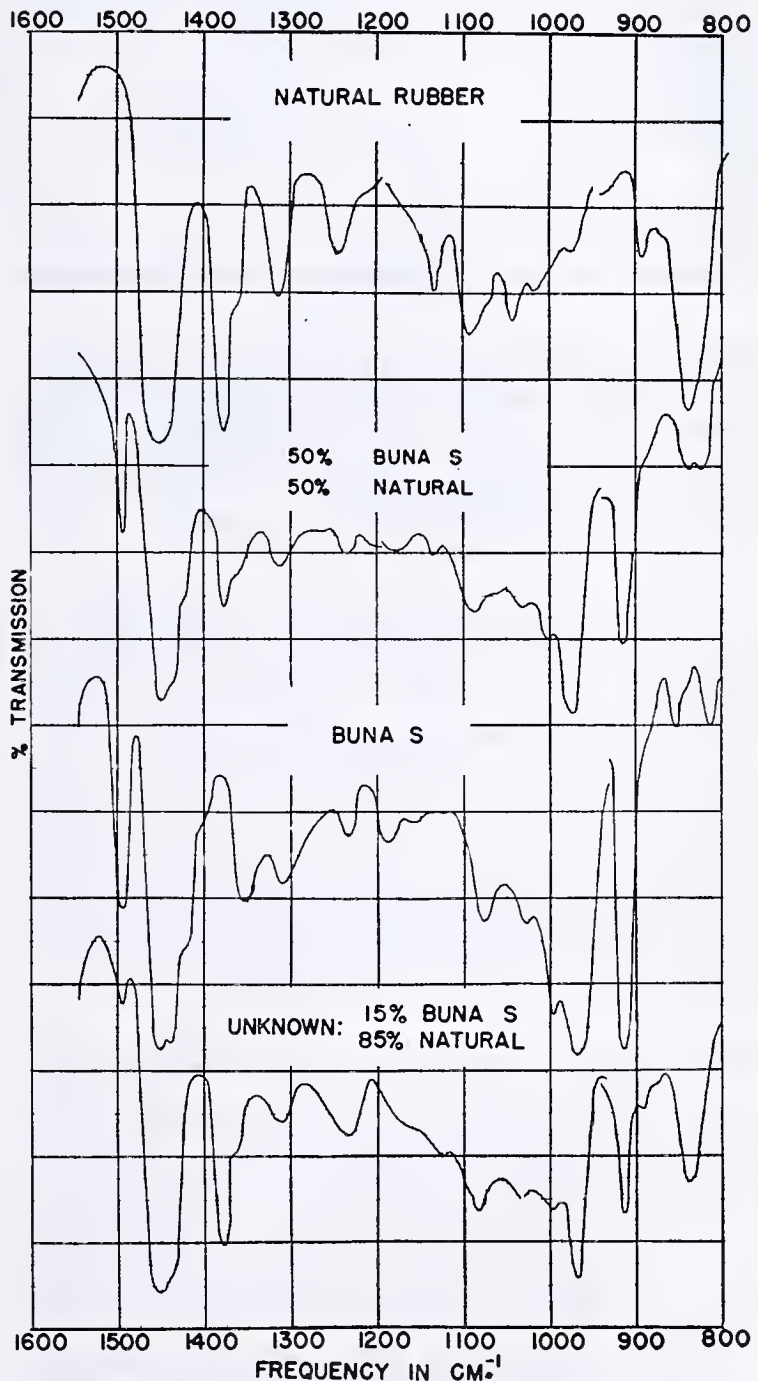


Figure 4. Outline of Infrared Analytical Method  
Spectra of prepared standards and unknown sample



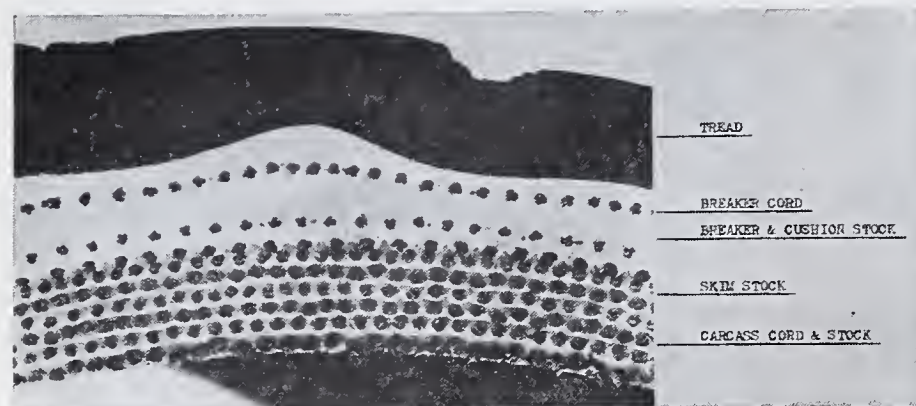


Figure 5. First Step in Sampling Procedure  
Labeled cross section of tire to be analyzed for rubber content

Table III. Composition of Typical Rubber Stocks

	87-1	Stocks A-912	A1-141
Smoked sheets	50.	100.	
Buna S	50.		100.
Zinc oxide	50.	5.	5.
Carbon black		50.	50.
Stearic acid	1.	2.5	
Pine tar	1.5	1.5	
Thermoflex A		1.4	
Neozone D		0.6	
Bardol			5.
Sulfur	2.	3.	2.
Captax		1.2	1.5
Altax	1.25		
Agerite resin D	1.5		

hot plate under reflux at 150° to 160° C. until the rubber stock is completely dissolved. Three to 4 hours are usually sufficient.

Cool and dilute with 20 cc. of benzene and then with 150 cc. of hexane. Allow the carbon black and other pigments to settle for at least 10 minutes, then decant the clear supernatant liquid through a No. 1 filter paper. If desired, filtration may be done with suction, using an asbestos pad.

Wash the flask, pigments, and filter with a mixture of 5 cc. of benzene and 45 cc. of hexane.

The above treatment easily removes the carbon black and other opaque compounding ingredients, giving a clear solution of the rubber from which the solvent may be removed by vacuum distillation.

In order to make valid comparisons between known and unknown samples, many mixtures of natural and Buna S rubber in varying proportions were compounded according to the typical methods of preparing tread, carcass, and tube stocks. The rubber content of these known samples was then extracted according to the final method discussed above and was used for infrared comparison. The compounding ingredients used in three such typical standards are given in Table III. The infrared absorption spectrum of the 50-50 mixture is shown in Figure 4.

#### TYPICAL ANALYTICAL RESULTS ON ACTUAL TIRE AND TUBE STOCKS

This entire project was initiated in order to provide a suitable method for determining the composition of tires and tubes, with particular reference to the types and relative amounts of the various rubbers present. The analytical methods which were evolved were applied successfully to a large number of samples. The type of information obtained is well illustrated by the results shown below, which were selected at random from the samples examined.

The tires, when received, were cross-sectioned, photographed, and labeled as shown in Figure 5, thus ensuring a permanent record of the tires and a ready means of identifying the various samples chosen. Wherever possible, samples of the tread,

cushion, breaker, and carcass stocks were removed from each tire, extracted, prepared for study, and then subjected to the two analytical procedures.

In Table IV, the results obtained on a few typical German tires and inner tubes are presented. In view of the spread in the phosphorus contents of natural rubbers from various sources (Table II), a mean value of 400 p.p.m. was used in making the analytical calculations from phosphorus determinations. The agreement between the infrared and the phosphorus methods is therefore all the more gratifying.

#### ACKNOWLEDGMENT

In conclusion, the authors would like to express their appreciation to the several other members of the staff of this laboratory who helped materially in the establishment of these analytical methods.

Table IV. Analysis of Captured German Tires

Tire No.	Phosphorus		Infrared	
	Natural	Natural	Buna S	
	P.p.m.	%	%	%
Tread Stocks				
FT 61	280	75	100	0
99	30	0	0	100
100	20	0	0	100
101	10	0	0	100
102	10	0	0	100
103	30	0	0	100
104	25	0	0	100
105	20	0	0	100
106	27	0	0	100
107	3	0	0	100
Carcass Stocks				
FT 61	340	100	100	0
99	340	100	100	0
100	380	100	100	0
101	430	100	100	0
102	130	25	20	80
103	190	50	50	50
104	230	60	50	50
105	200	50	80	20
106	305	80	100	0
107	190	50	50	50
Cushion and Tubes				
FT 61 <sup>a</sup>	290	75	100	0
99 <sup>a</sup>	270	70	100	0
101 <sup>a</sup>	450	100	...	...
102 <sup>b</sup>	200	50	75	25
103 <sup>b</sup>	255	65	75	25
104 <sup>b</sup>	285	70	85	15
105 <sup>b</sup>	295	75	100	0
106 <sup>b</sup>	260	70	100	0
107 <sup>b</sup>	290	75	100	0

<sup>a</sup> Cushion.

<sup>b</sup> Tubes.

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# Determination of Rate of Cure for Natural and Synthetic Rubber

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The tensile strength at a given marked undercure divided by the maximum tensile appears to be a convenient index of rate of cure. This index is called the tensile ratio. In comparing two stocks, the one having the higher tensile ratio is the faster curing. The proper undercure to use in calculating the tensile ratio has been found to be in the range of roughly 60% of the maximum tensile, which for many stocks occurs at about one fourth the time to reach maximum tensile. The tensile ratio is very easy to determine, requires no special equipment, and can be determined fairly accurately. In addition, it agrees well with generally accepted indexes of cure rate for typical natural rubber and GR-S formulations.

THE methods that have been proposed for determining the rate of cure of natural rubber stocks include:

Time to reach some maximum physical property, such as tensile strength, tensile product (12, 16), modulus at a definite strain, aged tensile, or some property of practical importance for a given compound.

Tests depending on temperature susceptibility like T-50 (5, 3, 7, 11, 14) or zero degree set (3).

Chemical tests, such as determination of the amount of combined sulfur (12).

Special tests based on physical measurements, such as time to reach the break in the modulus at a given strain vs. cure-time curve (4), time to reach the cure giving "reasonable snap with substantially unimpaired tear" (optimum hand tear method) (2), and time to reach minimum reduced residual elongation (8).

Of these methods, the T-50 test and the time to reach maximum tensile have been most widely accepted and have proved very useful, but leave something to be desired. The T-50 test requires special equipment not always available in smaller rubber laboratories, while the time to reach maximum tensile, although obtainable from tensile data which in most cases would have to be determined anyhow in order to evaluate the compound, is difficult to determine accurately, as the tensile vs. cure-time curve usually has a flat maximum.

In GR-S the need for a convenient index of rate of cure is more urgent. The T-50 test is not applicable to GR-S compounds (9) and as a rule the tensile vs. cure-time curve has an even flatter maximum than in rubber, so that the accuracy of determining the time to reach maximum tensile is even lower.

In considering the question of obtaining a satisfactory index of rate of cure, it will be helpful to define the terms "optimum cure", and "state of cure", "rate of cure".

Optimum cure for a given physical property may be defined as the time required to reach the maximum or optimum value for that property—for example, the optimum cure with respect to rebound would be the cure time at a specified cure temperature to bring the stock to its maximum rebound. The property most generally used is tensile strength and the optimum cure for tensile strength is often referred to simply as the optimum cure.

State of cure has been defined as the position of the cure in question in a series of cures (15). It seems more precise to define state of cure with respect to a given physical property as that fraction of the maximum value of the property shown by the cure in question (16). For example, if the 30-minute cure of a certain tread stock has a rebound of 47% and if the maximum rebound for this tread stock is 50%, then the state of cure of the 30-minute cure with respect to rebound can be expressed as  $47/50 = 0.94$ . It may be useful in some instances to distinguish undercures from overcures; we may, therefore, indicate overcures by placing a negative sign before the state of cure.

Rate of cure may be defined as the time required to reach a given state of cure compared to some standard or control stock. Specifically, rate of cure referred to a given state of cure equals time to reach that state for the control divided by the time for the sample to reach the same state. In the previous example, if the standard stock reaches a state of cure of 0.94 based on rebound in 20 minutes, the rate of cure of the sample tread stock would be  $20/30 = 0.67$ , since the sample stock required 30 minutes to reach the 0.94 state.

Optimum cure and state of cure depend on the physical property used to determine curing characteristics. Rate of cure may depend in addition on the state of cure at which the rate is measured. From a practical viewpoint the basic physical property is the useful life of a satisfactory commercial product made from the compound in question. If we then take useful life as the physical property for determining state of cure and use a state of cure of 1.0 (optimum cure) at which to measure rate of cure, we arrive at the following definition of rate of cure at a given

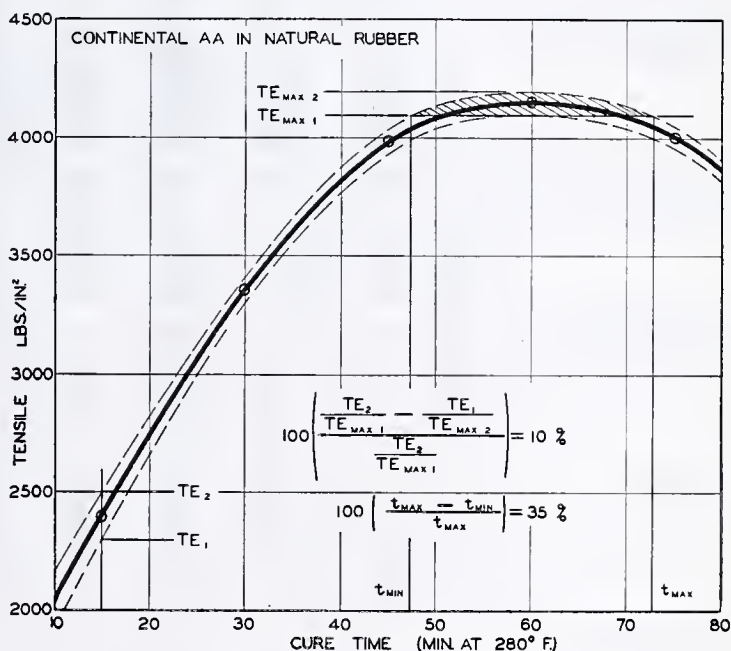


Figure 1

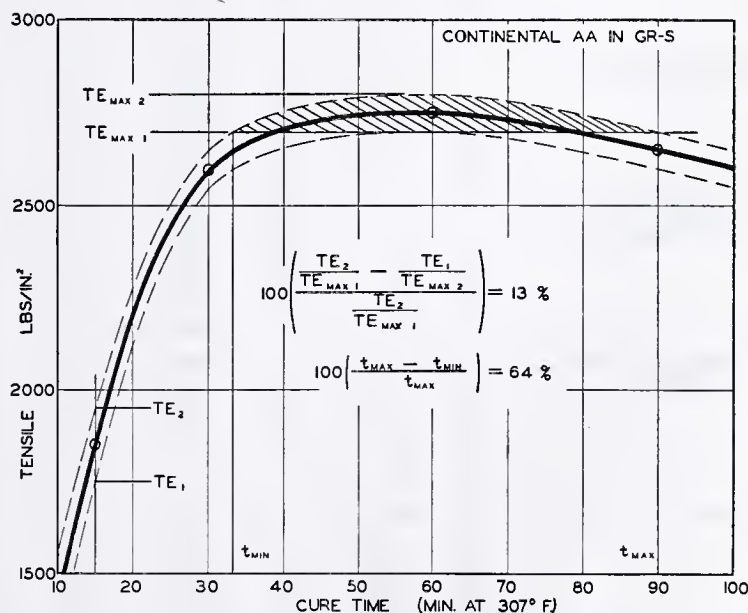


Figure 2



cure temperature—rate of cure for any stock is the cure time required to reach maximum useful life for a standard control stock divided by the cure time required to reach maximum useful life for the sample stock.

While the foregoing definition is precise, it is not very useful in actual laboratory work. Some index of rate of cure is therefore desired which correlates closely with rate of cure as defined above

but can be determined conveniently and accurately. In the absence of data connecting any proposed index of rate of cure with actual useful life tests it may be considered satisfactory to show that the proposed index correlates closely with indexes of rate of cure, the usefulness of which has already been established.

#### TENSILE RATIO

In order to determine which physical properties most closely fulfilled the above criteria for a useful index of rate of cure, the indexes suggested by previous investigators and other combinations of physical properties mentioned below were examined in natural rubber and GR-S. The best index was found to be the tensile ratio, which is defined as the tensile strength at a marked undercure divided by the maximum tensile strength. The undercure best suited for this purpose was found to be the cure giving a tensile strength of roughly 60% of the maximum tensile. For many tread type stocks the desired cure is roughly one fourth the time to reach maximum tensile. For example, for a series of stocks of the type which reach maximum tensile in about 60 minutes—say between 30 and 90 minutes—the proper undercure for calculating the tensile ratio would be about a 15-minute cure.

A very definite objection to the use of the time to reach some maximum property such as maximum tensile as an index of rate of cure is the inaccuracy involved in determining this time when the tensile cure-time curve has a flat maximum. The tensile ratio is, of course, not subject to this difficulty, as the flatter the maximum in the tensile vs. cure-time curve, the easier it is to determine accurately the value of the maximum tensile and hence the tensile ratio.

In Figures 1 and 2 the effect of the form of the tensile maximum on the accuracy of determining the time to reach maximum tensile is illustrated. In both figures the solid line gives the tensile strength as determined from the average of four identical stocks cured simultaneously at the indicated cures. From the deviations of the individual stocks from the average, the probable error (0.7 times the standard deviation) at each cure was calculated and the two broken lines correspond to the average value minus the probable error at the average plus the probable error.

Table I. Natural Rubber

Formula	Gum	Pptd. Whiting	MT	Conti-nex	Conti-nental AA	Conti-nental D	Conti-nental R-40
Smoked sheet	100	100	100	100	100	100	100
Zinc oxide	7.85	7.85	7.85	7.85	7.85	7.85	7.85
Pine tar	3	3	3	3	3	3	3
Stearic acid	3.3	3.3	3.3	3.3	3.3	3.3	3.3
Sulfur	2.81	2.81	2.81	2.81	2.81	2.81	2.81
Mercaptobenzothiazole	0.743	0.743	0.743	0.743	0.743	0.743	0.743
Precipitated whiting	.....	78	.....	.....	.....	.....	.....
Medium thermal (MT)	.....	.....	50	.....	.....	.....	.....
Continex (SRF)	.....	.....	.....	50	.....	.....	.....
Continental AA (EPC)	.....	.....	.....	.....	50	.....	.....
Continental D (MPC)	.....	.....	.....	.....	.....	50	.....
Continental R-40 (CC)	.....	.....	.....	.....	.....	.....	50

Cure at 280° F.							
Min.							
Modulus at 400% elongation, lb./sq. in.	0	.....	.....	.....	.....	.....	.....
	8	.....	340	300	900	350	250
	15	180	870	720	1420	1070	720
	30	280	960	1060	1920	1620	1060
	60	300	1100	1250	2120	2100	1430
	90	390	900	1350	2200	2330	2360
	180	390	780	1320	2300	2675	2630
Tensile at break, lb./sq. in.	0	0	100	70	200	250	370
	8	.....	.....	1620	1950	600	750
	15	2410	2450	2650	2900	2400	2040
	30	3580	2400	2700	3200	3340	2660
	60	3800	2300	2820	3340	3750	3290
	90	3490	2175	2550	3200	3900	3880
	180	2670	2000	2450	2450	3750	3780
Elongation at break, %	0	.....	975	900	750	860	975
	8	.....	.....	.....	600	775	800
	15	800	610	670	600	600	650
	30	765	600	600	550	600	640
	60	755	580	585	540	600	630
	90	695	580	550	450	565	570
	180	680	570	550	450	530	535
Breaking set, %	0	135	145	185	180	220	205
	8	.....	.....	.....	10	120	105
	15	7	15	17	12	17	24
	30	10	17	17	16	25	25
	60	10	12	22	21	30	27
	90	5	17	15	7	27	32
	180	0	15	11	7	21	25
Tear, lb./in.	0	.....	25	25	35	40	45
	8	170	100	100	150	65	90
	15	180	260	300	480	440	135
	30	220	250	325	485	800	250
	60	225	285	275	485	865	600
	90	180	200	230	370	770	730
	180	170	200	230	300	635	730
Durometer at 25° C.	0	24	34	30	37	40	39
	8	26	39	38	47	45	45
	15	34	48	45	54	52	51
	30	39	54	50	58	58	60
	60	41	56	51	60	60	62
	90	42	55	53	62	65	69
	180	41	55	51	62	69	70
Durometer at 100° C.	0	8	15	14	18	21	20
	8	35	44	38	40	28	28
	15	36	50	45	49	42	43
	30	41	56	50	56	56	54
	60	41	57	52	58	59	55
	90	42	55	52	61	63	65
	180	40	53	49	60	65	67
Rebound at 25° C., % Bashore	0	46	35	46	43	31	30
	8	49	39	51	38	31	32
	15	51	42	52	42	30	33
	30	57	52	55	48	33	34
	60	60	55	58	47	37	32
	90	58	53	59	47	36	32
	180	56	53	58	45	34	32
Rebound at 100° C., % Bashore	0	28	27	28	26	23	23
	8	54	58	60	48	33	34
	15	63	65	62	53	43	40
	30	70	69	73	63	48	48
	60	75	71	75	60	52	46
	90	68	68	77	61	50	46
	180	65	67	74	60	47	47
T-50, ° C.	0	23.0	22.5	22.5	21.0	22.0	21.0
	8	5.0	7.0	11.5	12.5	.....	18.5
	15	-1.0	0.0	4.5	5.5	12.5	15.3
	30	-18.0	-12.0	-6.5	-6.5	1.5	3.0
	60	-46.5	-24.0	-22.8	-18.0	-9.3	-7.2
	90	-47.5	-27.0	-26.5	-22.0	-13.7	-10.0
	180	-47.8	-27.0	-26.5	-25.5	-19.0	-15.8



Thus the two broken lines represent the 50% confidence limits for the tensile at various cures. The stock used for this purpose in Figure 1 was Continental AA in the natural rubber formulation given in Table I, while in Figure 2 Continental AA in the GR-S formula given in Table II was used. For the possible tensile *vs.* cure-time curves that can be drawn within the broken lines, the maxima can be anywhere in the shaded areas, so that the time to reach maximum tensile can vary from  $t_{min.}$  to  $t_{max.}$  The extreme per cent variation is therefore, as shown on the figures, 5% for the rubber stock and 64% for the flatter GR-S curve. From the same figures the extreme per cent variation for the tensile ratio can also be calculated as indicated on the figures and is 0% for rubber and 13% for GR-S. (These values are only approximate and illustrative. Since the experimental errors responsible for the variation in the individual curves are not entirely independent, the confidence limits for the various calculations may be somewhat different and the figures should not be considered as indicating a precise comparison of the accuracy of the tensile ratio and time to reach maximum tensile.)

#### DETERMINATION OF RATE OF CURE OF NATURAL RUBBER

In order to determine how satisfactory an index of rate of cure was furnished by the tensile ratio, various pigments were milled into a typical natural rubber formula, 26 volumes of pigment

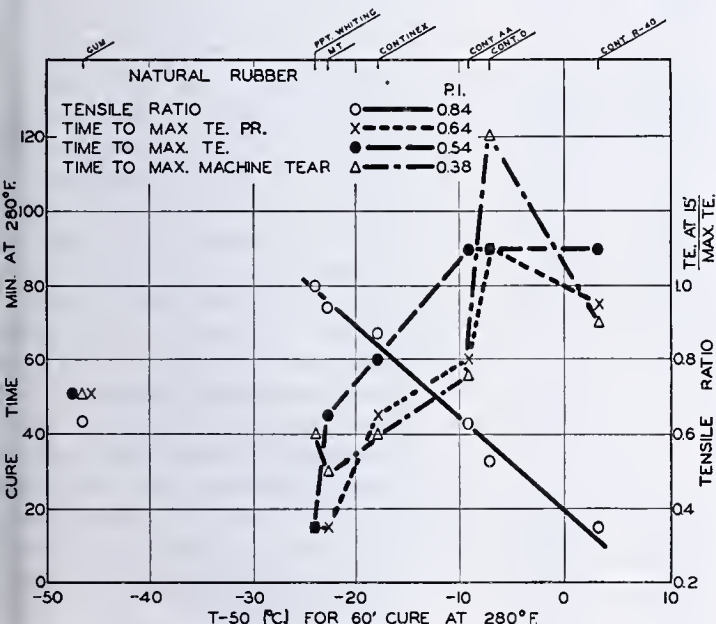


Figure 3

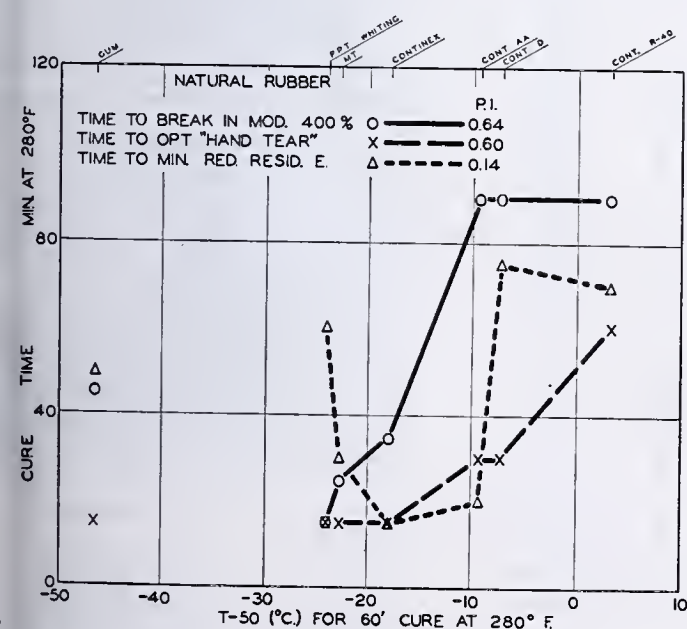


Figure 4

per hundred volumes of rubber being used in all cases. In Table I the formulas of the stocks used and the experimental test results are given. The results include tests on uncured stock and on a range of cures from 8 to 180 minutes and permit comparison of the tensile ratio and various indexes of rate of cure which have been used or recommended for natural rubber. The time to reach maximum tensile, tensile product (12, 16) (tensile multiplied by elongation), tear, and rebound were determined from the data. Likewise the time to reach minimum reduced residual elongation (breaking set divided by tensile) (8), break in modulus at 400% elongation (4), and optimum cure as determined by hand tear (2) were determined. In addition to tabulating the time to reach maximum values the time to reach various percentages (60, 70, 75, 80, 85, 90) of maximum values was determined for tensile strength and also for the other physicals.

We have defined state of cure as the ratio of the physical property at a given cure divided by the maximum value of the physical property. It was therefore thought of interest to see if the state of cure for any given cure, such as 15 minutes, could offer a useful index of rate of cure. For this purpose the ratio of tensile at all undercures to maximum tensile was determined and similar ratios for tensile product, rebound, modulus, and durometer. (In the case of modulus and durometer the 180-minute cure was taken as the maximum value.) Since the rebound of the uncured stock is appreciable, the ratio of the increase in rebound over uncured rebound at all undercures to the increase in rebound over uncured rebound for the maximum rebound was also calculated.

Finally, it was thought, the slope of the curve for the various physical properties *vs.* cure time at marked undercures might furnish an index of cure. Therefore the difference in tensile for various undercures—e.g., tensile at 15 minutes minus tensile at 8 minutes—divided by maximum tensile was tabulated and similar ratios for tensile product, tear, and rebound.

Inasmuch as the T-50 test is probably the most generally accepted index of rate of cure for natural rubber, the indexes enumerated above were tested by determining their correlation with T-50. The tensile ratio which for this series of stocks was taken as the tensile at 15 minutes divided by the maximum tensile was found to give the best correlation.

The results are summarized in Figures 3 and 4, where the tensile ratio and other indexes of rate of cure are plotted against T-50 at the 60-minute cure. The other indexes are those found to give the closest correlation with T-50 or which have been previously suggested in the literature.

The prediction indexes (P.I.) indicating the degree of correlation between the various indexes of rate of cure and T-50 are listed on the figures. (Prediction index =  $1 - \sqrt{1 - r^2}$ , where  $r$  is the coefficient of correlation calculated according to standard statistical methods. P.I. is 0 for no correlation and 1 for perfect correlation. For six pairs of values as in the present instance  $r > 0.8$  or P.I.  $> 0.4$  shows significant correlation within the 95% confidence limits.) The prediction index of 0.84 for tensile ratio was the highest found. Likewise the fact that tensile ratio is the only function which can be fairly well approximated by a single straight line confirms the indication given by the prediction indexes.

The tensile ratio has been used in the authors' laboratory as an index of rate of cure for various channel black stocks. In a series of twenty-seven experimental blacks in the same base formula used for the pigments in Table I the tensile ratio and time to reach maximum tensile were both measured. The rate of cure as defined previously was then determined by dividing the tensile ratio of each experimental stock by the tensile ratio for the standard control which was cured at the same time as the experimental sample. The same control was, of course, used throughout the series. Likewise, the rate of cure was also estimated by dividing the time to reach maximum tensile of the control by the time to reach maximum tensile for the sample. The







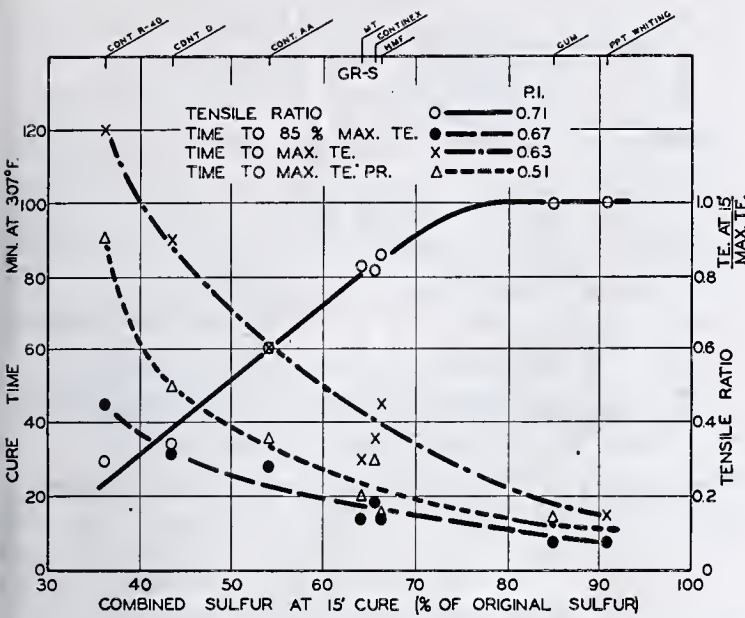


Figure 5

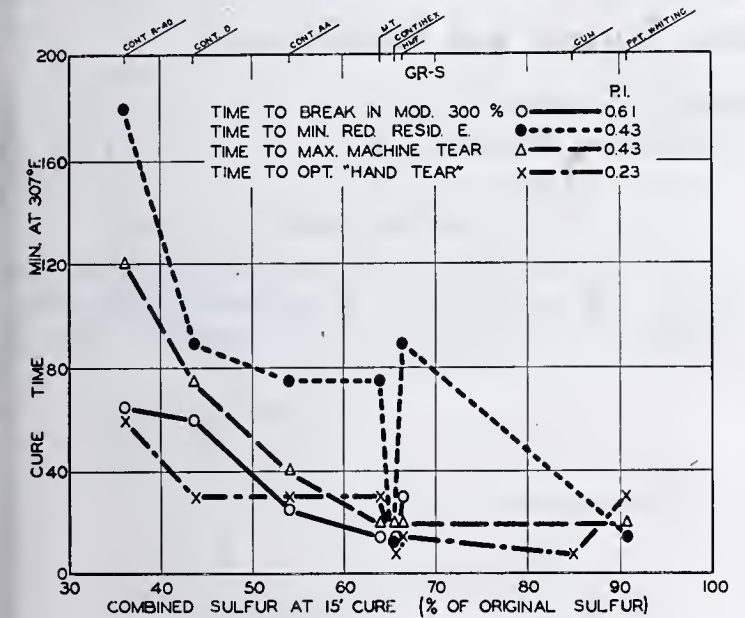


Figure 6

the combined sulfur at the 15-minute cure expressed as per cent of original sulfur. An index analogous to tensile ratio but utilizing tensile product instead of tensile was also found to correlate with combined sulfur almost as well as tensile ratio but was not included in the graph.

Prediction indexes listed on the figures indicate the best correlation for tensile ratio, time to reach 85% of maximum tensile, time to reach maximum tensile, and time to reach break in modulus. The highest value for the prediction index is obtained with tensile ratio.

Since rate of cure increases as temperature of cure is increased, a good test of an index of rate of cure is the behavior of the index at different cure temperatures. In Figure 7, tensile ratio, combined sulfur at the 15-minute cure, and time to reach maximum tensile are plotted against cure temperature. For these tests Continental AA in the GR-S formula given in Table II was used. In addition to the stocks cured at various temperatures, four duplicate stocks were cured at 307° F. in order to give some indication of the reproducibility of these three methods.

Prediction indexes giving the correlation of the indexes of cure rate with cure temperature are listed at the top of Figure 7. The best correlation with cure temperature is obtained with tensile ratio and combined sulfur. Time to reach maximum tensile also shows a significant correlation with cure temperature but does not correlate as closely as the other two indexes. (For the eight stocks used in this case a prediction index > 0.3 or a correlation coefficient > 0.7 indicates a significant correlation within 95% confidence limits.)

DISCUSSION

Studies with GR-I stocks also indicate that the tensile ratio is a useful index of cure for this synthetic. It is planned to present these results in a separate paper.

In dealing with certain special problems the time required to obtain a satisfactory index of rate of cure may possibly be shortened. For example, in comparing a series of stocks all of which have very similar maximum tensiles, the tensile of the proper undercure is approximately proportional to the tensile ratio and can be used similarly as an index of rate of cure. A case of this sort would occur in the control testing of rubber channel blacks or other pigments. On the other hand, although the maximum tensiles for different stocks in a series may be different, the cure rate may be sufficiently similar so that the tensile of a fixed cure in the maximum tensile range, say the 60-minute cure, may be fairly close to the maximum tensile for all stocks. In this event the tensile ratio would be approximately equal to the tensile at 15 minutes/tensile at 60 minutes, which could then be used as an index of rate of cure and would only require the making of two cures. A possible example of this type might be a series of stocks containing different amounts of petroleum-type softeners.

The rate-of-cure results for both natural rubber and GR-S indicate that the tensile ratio is a satisfactory index of rate of cure even in a series of stocks containing a widely different variety of pigments. However, it seems likely that the greatest usefulness for the tensile ratio will occur in the comparison of similar pigments or other compounding ingredients such as carbon blacks, inorganic pigments, softeners, antioxidants, etc., in the same test formula.

The above short cuts as well as tensile ratio itself are satisfactory indexes of rate of cure only within a set of stocks milled and cured side by side, such as the stocks in Tables I and II. In the comparison of stocks not milled and cured together a reference control is customarily run at the same time as each stock. The

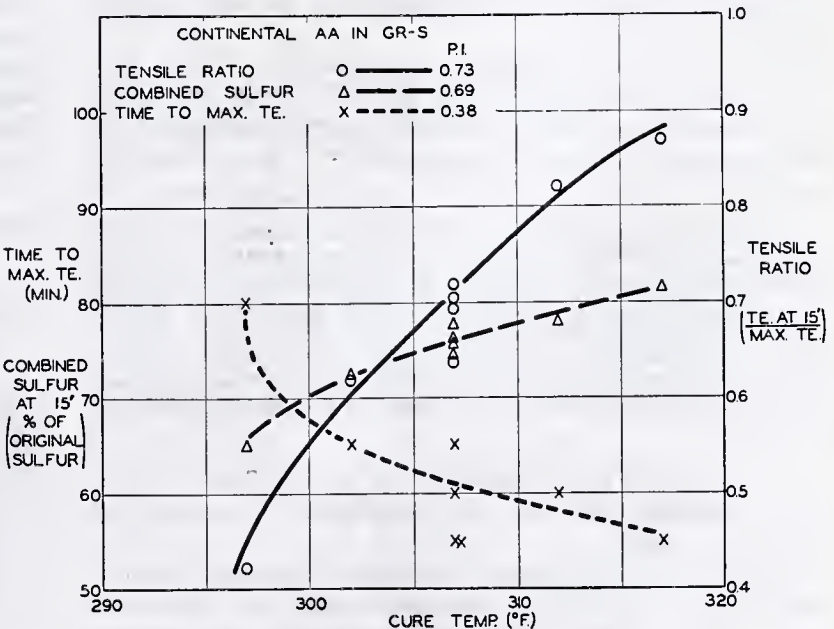


Figure 7



tensile ratio of the individual stocks divided by the tensile ratio for the corresponding control should then be taken as the index of rate of cure.

#### ACKNOWLEDGMENT

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## Determination of Alpha,Para-Dimethylstyrene In the Presence of Para-Methylstyrene, Styrene, and Para-Cymene

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$\alpha$ , $p$ -Dimethylstyrene can be determined in the presence of  $p$ -methylstyrene, styrene, and  $p$ -cymene by two independent methods. The chemical method depends upon addition of hydrogen chloride or bromide to the styrenes with subsequent estimation of the tertiary halide formed by  $\alpha$ , $p$ -dimethylstyrene. The other method involves an analysis of the ultraviolet absorption curve of such mixtures. Results obtained by these independent methods are in good agreement.

NEED has recently arisen for a method for the determination of  $\alpha$ , $p$ -dimethylstyrene in the presence of  $p$ -methylstyrene, styrene, and  $p$ -cymene. Since no satisfactory procedures were available for the analysis of such mixtures, chemical methods, ultraviolet absorption, and polarographic analysis were considered.

Preliminary experiments on the last, using the technique of Laitinen and Wawzonek (1) were not promising and this approach was not studied further.

It was found possible, however, to analyze such mixtures by a chemical method and also by means of ultraviolet absorption. Both procedures were readily adaptable to control use and have been successfully applied to the analysis of a large number of samples.

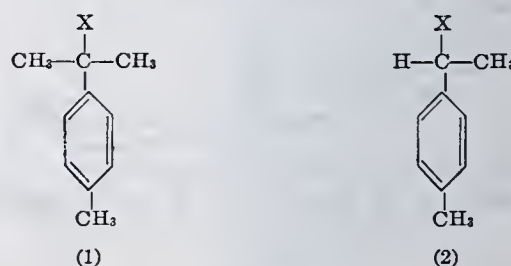
In order to test these methods it was first necessary to obtain pure samples of the various styrenes and  $p$ -cymene. The  $\alpha$ , $p$ -dimethylstyrene and  $p$ -methylstyrene were synthesized and purified in this laboratory. A good grade of commercial styrene was vacuum-distilled several times before use. The  $p$ -cymene was distilled through a 40-plate column packed with 0.23-cm. ( $3/32$ -inch) stainless steel helices at 100-mm. pressure and a reflux ratio of approximately 60/1, a middle cut being collected and used in this work.

The constants of these compounds are given in Table I. The bromine numbers were run by a modification of the method of Uhrig and Levin (9).

The refractive index results are in good agreement with the International Critical Tables values, the temperature being considered. The samples of the various styrenes were always freshly vacuum-distilled before being used.

#### CHEMICAL METHOD

The chemical method of analysis depends upon the following reaction. If the addition of HX to  $\alpha$ , $p$ -dimethylstyrene and  $p$ -methylstyrene follows Markownikoff's rule, where X is either Br or Cl, the following compounds will be formed:



The halogen in (1), formed from  $\alpha$ , $p$ -dimethylstyrene, is attached to a tertiary carbon atom, while that in (2), formed from  $p$ -methylstyrene, is attached to a secondary carbon atom. The tertiary halogen in (1) is much more readily hydrolyzed than the secondary in (2), and this fact offers a means of analytically determining  $\alpha$ , $p$ -dimethylstyrene in the presence of  $p$ -methylstyrene and styrene. There is no reaction of HX with  $p$ -cymene.

It has been reported that hydrogen bromide adds rapidly to styrene (2, 10), and that the secondary bromide formed by nor-

Table I. Constants of Materials Used in Testing Methods

Compound	Formula	$n_D^{20}$	$n_D$ (I.C.T.)	Bromine No. Observed	Theory
$p$ -Cymene	$p\text{-CH}_3\text{C}_6\text{H}_4\text{CH}(\text{CH}_3)_2$	1.4913	1.4947 (15° C.)	<1	0
Styrene	$\text{C}_6\text{H}_5\text{CH}=\text{CH}_2$	1.5467	1.5467 (20° C.) <sup>a</sup>	151	154
$p$ -Methylstyrene	$p\text{-CH}_3\text{C}_6\text{H}_4\text{CH}=\text{CH}_2$	1.5421	1.5447 (16.4° C.)	137	135
$\alpha$ , $p$ -Dimethylstyrene	$p\text{-CH}_3\text{C}_6\text{H}_4\text{C}(\text{CH}_3)_2$	1.5356	1.5344 (18.7° C.)	117	121

<sup>a</sup> Value of Moore, Burk, and Lankelma (7).



**Table II. Determination of  $\alpha,p$ -Dimethylstyrene in Known Mixtures**

Other Materials Present	(Using method involving addition of HBr) Weight of $\alpha,p$ -Dimethylstyrene		Recovery %
	Present Gram	Recovered Gram	
None	0.2731	0.2545	93.3
None	0.2131	0.1977	92.8
0.2080 gram <i>p</i> -methylstyrene	0.2408	0.2215	91.9
None	0.2693	0.2477	92.0
None	0.2414	0.2245	94.0
0.1 ml. of benzene, 0.2 ml. of styrene, 0.2 ml. of <i>p</i> -methylstyrene, 0.5 ml. of <i>p</i> -cymene	0.4147	0.3942	95.2
	0.2613	0.2522	96.7
25.6% <i>p</i> -methylstyrene and 48.8% <i>p</i> -cymene	0.0854	0.0796	94.2
	0.1006	0.0926	92.0

mal addition may be hydrolyzed under relatively mild conditions, indicating that the conditions of hydrolysis of the tertiary halide must be chosen so as to avoid interference from the secondary halides present. Based on these facts, the following methods have been developed, involving the addition of HX, removal of excess, followed by hydrolysis, and estimation of the tertiary halide formed from  $\alpha,p$ -dimethylstyrene under conditions where there is no appreciable hydrolysis of the secondary halide formed from *p*-methylstyrene and styrene.

**HYDROGEN BROMIDE METHOD.** A sample weighing approximately 0.5 to 1.0 gram is accurately weighed out into a 125-ml. Erlenmeyer flask containing 25 ml. of carbon tetrachloride. Gaseous hydrogen bromide, generated by dropping bromine onto naphthalene and purified by passing through several towers of naphthalene and finally one containing Drierite, is bubbled through the sample for 30 minutes at a rate of 3 to 4 bubbles per second. At the end of this time the addition tube is washed down with 10 ml. of carbon tetrachloride and a brisk stream of nitrogen is passed through the solution for 30 minutes to remove unreacted hydrogen bromide. The flask is then removed and chilled in an ice-salt bath for 10 minutes. Thirty milliliters of

previously chilled 90% alcohol are then added, the flask is swirled for a few seconds, two drops of methyl red indicator are added, and the cold solution is rapidly titrated with standard 0.1*N* alcoholic potassium hydroxide to a bright yellow end point lasting for 10 seconds. Table II gives the results obtained by this method on known samples.

**HYDROGEN CHLORIDE METHOD.** The procedure is the same as that used in the hydrogen bromide method with the following changes:

Benzene instead of carbon tetrachloride is used as the solvent.

Forty milliliters of 80% alcohol are used to effect hydrolysis of the tertiary halide.

The titration is performed at room temperature and the sample titrated with 0.1*N* alcoholic potassium hydroxide to a 30-second bright yellow end point. Hydrogen chloride was generated by dropping concentrated hydrochloric acid into concentrated sulfuric acid. The evolved gas was passed through a tower filled with concentrated sulfuric acid before being passed into the solution being analyzed.

Table III gives the results obtained by this method on known samples. The end points are considerably sharper than those given by the hydrogen bromide method.

**Table III. Determination of  $\alpha,p$ -Dimethylstyrene in Known Mixtures**

Substances Present	(Using method involving addition of HCl) Weight of $\alpha,p$ -Dimethylstyrene		Recovery %
	Present Gram	Recovered Gram	
<i>p</i> -Cymene	None	None	...
Styrene	None	None	...
<i>p</i> -Methylstyrene	None	None	...
$\alpha,p$ -Dimethylstyrene	0.2986	0.2740	93.5
	0.4739	0.4508	95.3
	0.4116	0.3903	94.7
	0.3077	0.2940	95.5 <sup>a</sup>
	0.5693	0.5415	95.3 <sup>a</sup>
$\alpha,p$ -Dimethylstyrene plus 0.5 ml. each of <i>p</i> -cymene, styrene, and <i>p</i> -methylstyrene	0.4015	0.3855	96.0
	0.4052	0.3902	96.2

<sup>a</sup> HCl gas added for 1 hour instead of 30 minutes as in all other cases.

**Table IV. Analyses by Hydrogen Bromide and Hydrogen Chloride Methods**

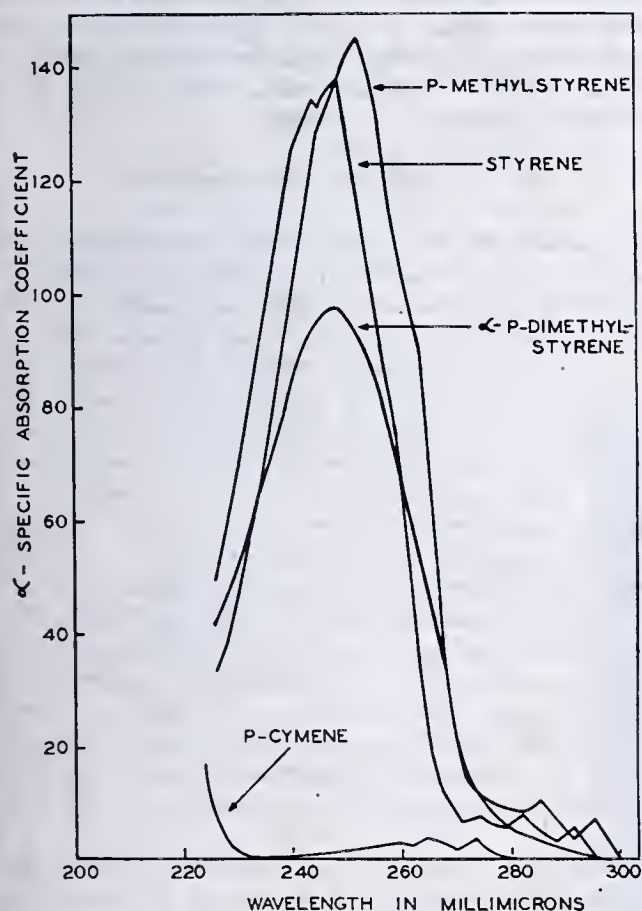
Sample	$\alpha,p$ -Dimethylstyrene	
	HBr method %	HCl method %
A	19.6, 20.2	19.8, 20.6
B	29.2, 29.5	28.2, 29.3
C	20.2, 19.9	20.2, 19.8
D	20.5, 20.4	20.6, 20.6
E	22.6, 22.0	22.6, 23.3
F	10.6, 10.7	9.5, 9.5
G	32.8, 32.6	32.2, 32.0
H	32.9, 32.6	31.8, 32.5

## DISCUSSION OF RESULTS

An examination of Tables II and III shows that somewhat low recoveries (about 95%) of  $\alpha,p$ -dimethylstyrene are obtained in every case. Therefore, to correct for this a factor of 1.05 is used in calculating the results. This low recovery of  $\alpha,p$ -dimethylstyrene is not raised by a longer time of addition of the hydrogen halide. It may be due to the fact that a small fraction of the addition takes place contrary to Markownikoff's rule. A consideration of the results obtained by use of these methods in the analysis of over fifty samples of known and unknown composition indicates that the precision of the methods is approximately  $\pm 3\%$  of the  $\alpha,p$ -dimethylstyrene present.

Table IV shows the agreement between the hydrogen bromide and hydrogen chloride methods in the analysis of eight different samples of unknown composition.

The agreement between the results obtained by the two methods is seen to be good. Since the hydrogen chloride method is somewhat easier to use and the end point is sharper, it is recommended for use.

**Figure 1. Ultraviolet Absorption Curves**

For  $\alpha,p$ -dimethylstyrene, *p*-methylstyrene, styrene, and *p*-cymene in ethanol solutions



## ULTRAVIOLET ABSORPTION METHOD

The spectrophotometric method has been successfully applied to the quantitative determination of compounds having characteristic absorption bands in the ultraviolet region of the spectrum. Good examples of this method used in calculating two, three, and four constituents of a mixture have been reported in the literature (3-6).

In mixtures of pure styrene,  $\alpha$ , $p$ -dimethylstyrene,  $p$ -methylstyrene, and  $p$ -cymene it was found possible to determine the amount of each styrene present within 2% of the known value.

The absorption data were obtained from measurements made with a Beckman quartz spectrophotometer. The solvent was ethanol in all cases. The formulas used in making the calculations use the term specific  $\alpha$ :

$$\text{Specific } \alpha = \frac{\log_{10} \frac{I_0}{I}}{cl}$$

where  $\alpha$  = absorption coefficient

$I_0$  = intensity of radiation transmitted by the solvent

$I$  = intensity of radiation transmitted by the solution

$c$  = concentration of solute in grams per 1000 ml.

$l$  = length in centimeters of solution through which the radiation passes

The samples of pure  $\alpha$ , $p$ -dimethylstyrene,  $p$ -methylstyrene, styrene, and  $p$ -cymene used to obtain the specific absorption coefficients of the pure compounds were the same as those used in the development of the chemical method previously described.

The values at different wave lengths are shown in Figure 1. The curve for styrene agrees very closely with previously published data concerning this compound (8).

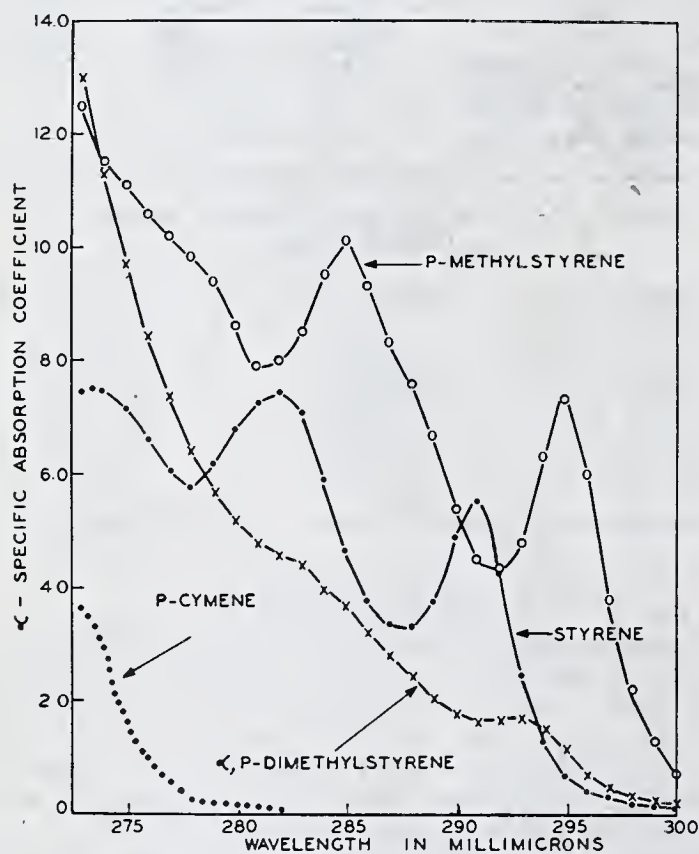


Figure 2. Ultraviolet Absorption Curves

For  $\alpha$ ,  $p$ -dimethylstyrene,  $p$ -methylstyrene, styrene and  $p$ -cymene in ethanol solutions (enlarged scale of spectral region 275 to 300  $m\mu$ )

The styrenes have a strong absorption band in the spectral region 248 to 252  $m\mu$  with less pronounced but yet distinct bands in the region to 300  $m\mu$ . For the three styrenes the bands in the region 248 to 252  $m\mu$  are so similar that no attempt was made to use them for quantitative determinations. A larger scale graph of the region 280 to 300  $m\mu$  (Figure 2), shows that at certain

Table V. Per Cent Composition

Mixture	Composition of Mixture of Pure Compounds	Known %	Calculated from Ultraviolet Absorption Data %	Error %
1	$\alpha$ , $p$ -Dimethylstyrene $p$ -Methylstyrene Styrene	32 42 26	30.8 42.4 26.0	1.2 0.4 0
2	$\alpha$ , $p$ -Dimethylstyrene $p$ -Methylstyrene Styrene	5 5 90	6.0 5.3 88.1	1.0 0.3 1.9
3	$\alpha$ , $p$ -Dimethylstyrene $p$ -Methylstyrene Styrene	5 90 5	3.9 90.3 5.3	1.1 0.3 0.3
4	$\alpha$ , $p$ -Dimethylstyrene $p$ -Methylstyrene Styrene $p$ -Cymene	25 26 25 24	24.3 26.3 25.9 ...	0.7 0.3 0.9 ...
5	$\alpha$ , $p$ -Dimethylstyrene $p$ -Methylstyrene Styrene $p$ -Cymene	20 30 35 15	21.9 29.4 35.8 ...	1.9 0.6 0.8 ...

Table VI. Samples of Unknown Composition

Sample	$\alpha$ , $p$ -Dimethylstyrene Chemical method %	Ultraviolet absorption method %
1	23.0, 22.9	21.7, 22.3
2	20.0, 20.2	19.8, 20.3
3	32.5, 32.3	32.8, 32.5
4	27.3, 27.4	27.8, 27.5

wave lengths there are sufficient differences in  $\alpha$  of the pure compounds to permit quantitative calculations. It is also evident that  $p$ -cymene has its absorption values below 280  $m\mu$  and therefore does not appreciably affect absorption values above 280  $m\mu$ , even though present in quantities up to 70 or 80%.

Quantitative determinations on three unknowns require the selection of three suitable wave lengths in order to set up three simultaneous equations. At a given wave length, components  $a$ ,  $b$ , and  $c$  present in a solution in percentages of  $x$ ,  $y$ , and  $z$ , respectively, will give a total absorption value which can be represented by the equation:

$$\frac{(\alpha_a)x + (\alpha_b)y + (\alpha_c)z}{100} = \alpha_{\text{solution}}$$

$\alpha_a$ ,  $\alpha_b$ ,  $\alpha_c$  represent the specific absorption coefficients for each of the pure compounds at that particular wave length. By setting up three equations at different wave lengths, the percentages ( $x$ ,  $y$ , and  $z$ ) of each component can be calculated.

Since simultaneous equations with three or more unknowns are customarily solved by the method of determinants, the criterion for the selection of wave lengths is that the determinants in the expressions for the unknown concentrations be as large as possible. Likewise, the individual values of  $\alpha$  should be large enough to be measured with accuracy on the spectrophotometer.

In known mixtures of pure compounds, with or without  $p$ -cymene, it was found possible to calculate the per cent composition by using the absorption data at wave lengths 285, 291, and 295  $m\mu$  (Table V). Values calculated from the absorption data differ from known values by less than 2%. The presence of  $p$ -cymene did not interfere with the calculations. The wave lengths 283, 287, and 291  $m\mu$  were found to give just as accurate values as the previously mentioned wave lengths. It is therefore possible to use two sets of wave lengths to serve as checks on each other.

Four samples of unknown compositions were analyzed for  $\alpha$ , $p$ -dimethylstyrene by both the chemical and ultraviolet absorption methods (Table VI).

The agreement between these independent methods is seen to be good.



## ACKNOWLEDGMENTS

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# Determination of Soluble Pectin and Pectic Acid by Electrodeposition

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In a new approach to the determination of pectin, solutions of pectin or pectates are deashed by the use of ion-exchange resins. The pectin or pectic acid is electrolytically deposited at a platinum anode in a weighable form. The method requires less of the analyst's time and attention than does the calcium pectate method and, with partially purified solutions, gives results of the same order of accuracy. The method is especially applicable to amounts of pectin ranging between 5 and 50 mg.

INVESTIGATIONS designed to develop new and extended uses for pectin have made desirable an analytical method capable of determining small amounts of pectin. Approximate estimations of pectin can be made by precipitation with 70 to 80 per cent alcohol or 50 per cent acetone (1, 3, 8, 9). Alcohol and acetone of these strengths precipitate gums, some proteins, and calcium and potassium salts of some of the organic acids as well as pectin. However, when dilute solutions are used the results are likely to be low because of incomplete precipitation of the pectin and difficulties in handling small amounts of the precipitate. The alcohol precipitate is difficult to filter and wash thoroughly. This filtration may be improved by precipitation with acidified alcohol, but after several washings enough acid may remain with the pectin to cause charring when the precipitate is dried. Fifty per cent acetone yields a precipitate which is more easily filtered and washed but affords little improvement in accuracy.

The pectic acid method (1) is long and subject to error due to the solubility of the pectic acid. The calcium pectate method of Emmet and Carré (4), although tedious and time-consuming, is probably the most reliable of the methods in use at the present time, but is not readily adaptable to very small amounts of pectin because of the large number of manipulations required. It has been used throughout this investigation for the analysis of stock solutions for comparisons with the proposed method. The proposed method is the result of an attempt to devise an accurate method which requires less of the analyst's time and attention and can be used to determine smaller amounts of pectin than the method of Emmet and Carré.

Since the soluble pectinous materials are negatively charged colloids, it was decided to investigate the possibility of collecting the pectin on the anode of a suitably arranged electrolysis system. Brown (2) unsuccessfully attempted to precipitate pectin electrolytically from aqueous solutions containing large amounts of electrolytes. Griggs and Johnstin (5) observed flocculation of pectin at the anode on electrolysis with 110-volt direct current. The authors have found that pectin can be quantitatively determined by electrodeposition, provided the electrolyte concentration of the solution is low. Ion-exchange resins can be used when it is necessary to remove the electrolytes before electrodeposition.

## APPARATUS

A conventional electrolysis apparatus supplied with 220-volt direct current from a rectifier-transformer unit was used. A mercury cathode cell was constructed from a 250-ml. Griffin-type beaker, into the side of which was fused a platinum wire (Figure 1). A mercury-filled side arm was added to provide flexibility in the connection to the negative binding post. Clean mercury completely covering the platinum wire in the vessel served as the cathode. The anode was a disk of 45-mesh platinum gauze 6.25 cm. (2.5 inches) in diameter, edged with 0.075-cm. (0.03-inch) platinum wire to give rigidity and supported by a 15-cm. (6-inch) piece of 0.127-cm. (0.05-inch) platinum wire attached to the center (Figure 1). Similar electrodes are avail-

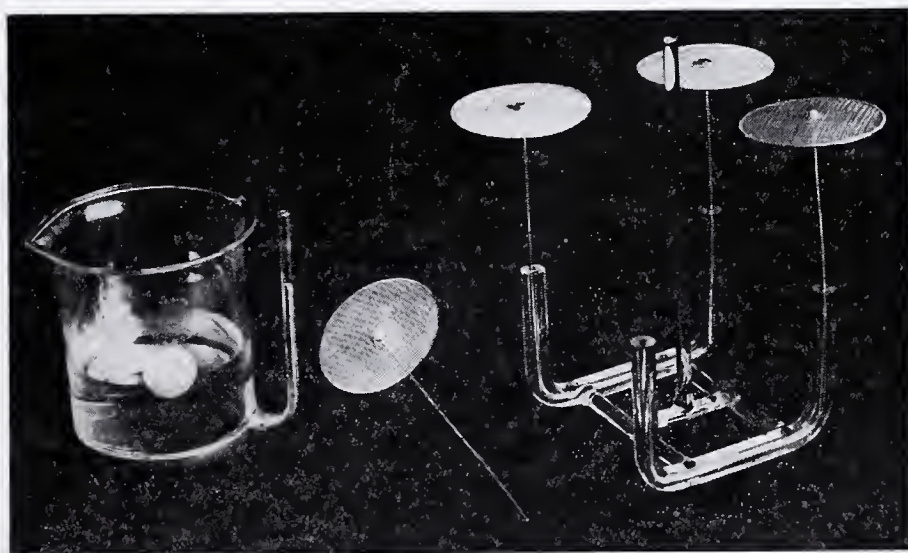


Figure 1. Electrolysis Vessel, Anode and Anodes in Holder



Table I. Electrodeposition of Pectin from Solutions Containing Various Percentages of Alcohol

(25-ml. aliquots of stock solution. Time, 5 hours)						
Alcohol Content % by volume	Weights of Deposits				Remarks	
	Mg.	Mg.	Mg.	Mg.		
30	17.2	18.9	20.6	..	Solutions became hot. One of 4 deposits did not adhere to anode	
40	21.8	22.1	22.2	21.9	Compact deposits	
50	7.6	7.9	8.5	5.7	Deposits not compact	
60	7.1	7.6	9.2	9.8	Bulky deposits	

Table II. Time Required for Electrodeposition of Pectin from 40 Per Cent Alcohol Solutions

(25-ml. aliquots of stock solution)						
Time Hr.	Weights of Deposits				Average	Remarks
	Mg.	Mg.	Mg.	Mg.		
2	19.9	20.4	19.4	19.0	19.7	Precision poor
3	18.9	20.5	19.6	20.3	19.8	Precision poor
4	20.8	20.3	20.1	20.1	20.3	Precision fair
5	21.8	22.1	22.2	21.9	22.0	Precision good
6	21.9	21.7	22.2	22.1	22.0	Precision good

able from platinum manufacturers. This electrode system has proved to be much more satisfactory than one using either cylindrical or spiral electrodes, since the mercury cathode-disk anode system makes it possible to have all the solution under the influence of the field without stirring.

Ion-exchange resin columns were prepared from 24 × 190 mm. glass tubing constricted to a 6-mm. outlet (Figure 2). Ten grams drained weight of ion-exchange resin, supported on a glass wool mat, was used in each tube. The cation-exchange column was mounted so that the effluent dripped directly into the acid-absorbing bed.

#### ELECTRODEPOSITION OF PECTIN FROM LOW-ASH SOLUTIONS

The following procedure was found to give a firm deposit that adhered very well to the anode:

An aliquot of the aqueous solution containing approximately 5 to 50 mg. of pectin was placed in the electrolysis vessel and diluted to 58 ml. with distilled water, and 42 ml. of 95 per cent ethyl alcohol were added with stirring. To obtain quantitative deposition in the shortest time, the alcohol concentration must be close to 40 per cent by volume, as indicated in Table I. Precipitation of the pectin at this point indicates that the electrolyte content is too high and that the solution must be deashed. During the electrolysis the vessel was immersed in a water-ice bath to prevent an undue rise in the temperature of the solution. The anode was lowered into the solution so that the gauze was barely covered and 220-volt direct current was applied. The current varied between 5 and 20 milliamperes, depending on the electrolyte content and the temperature of the solution. No stirring of any kind was employed during the run. Occasionally it was necessary to adjust the anode level if the solution had expanded or contracted as a result of changes in temperature. At the end of 5 hours the anode was withdrawn from the vessel and immersed in 99 per cent ethyl alcohol for 3 minutes and then in anhydrous ether for 3 minutes.

A very compact deposit was obtained, which was further dehydrated by the alcohol-ether treatment to a very thin uniform film. This alcohol-ether treatment permitted complete drying in one hour at 105° C. The electrodes were cleaned by immersion in boiling water for a few minutes, followed by rinsing in distilled water. If the deposit was pectic acid, the addition of a little dilute alkali aided in the solubilization. Removal of the deposit by ignition in a flame was avoided because of the possible danger of changing the electrode surface.

Table II shows the relation between the time of deposition and the weight of the deposit.

#### REMOVAL OF ELECTROLYTES FROM HIGH-ASH SOLUTIONS

Pectin deposition from extracts of fruit was unsuccessful because the current was transferred by the electrolytes present. The solution heated up and gas was evolved from both electrodes. No deposit was obtained.



Figure 2. Deashing System

Upper, cation exchange column,  
lower, acid adsorbing column

A system of commercial ion-exchange resins has been used to remove electrolytes from pectin solutions in this laboratory. (The use of exchange materials for the removal of ash constituents from pectin solutions was first investigated in this laboratory by W. D. Maclay and co-workers who are investigating the efficiencies of various commercial products for this purpose.) The IR 100 resin removes the cations by exchange with hydrogen ion, while the IR 4 resin removes the acids which would interfere, by conductance, with the electrodeposition of pectin. No pectin was lost during this process when the conditions described below were followed. The capacity and regeneration of these resins are described by the manufacturer (7).

Solutions containing 0.4 to 1.0 mg. of pectin per ml. were deashed in the following manner: The columns were rinsed with two 10- to 15-ml. portions of the solution, with thorough drainage between rinses. The rinsings were discarded and the main portion of the solution was deashed by

percolation through the columns at the rate of approximately 3 ml. per minute.

The pectin concentration of the solution was unchanged by this treatment (Table III). The pectin solutions treated in this manner were low in ash, ranging from 0.5 to 4 mg. per 100 ml., and successful electrodepositions could be made from such solutions. The resin system has been used successfully on neutral, acid, and alkaline aqueous extracts of pectin.

Table III. Pectin Content of Solutions before and after Ion-Exchange Treatment

Source of Pectin	Before <sup>a</sup>	After <sup>a</sup>
	Mg./ml.	Mg./ml.
Extract of whole grapefruit	1.03	1.03
Extract of whole orange	0.64	0.64
Extract of whole apple	0.41	0.40
High-ash commercial pectin	0.35	0.35

<sup>a</sup> Average of three analyses by method of Emmet and Carré (4).

Table IV. Electrodeposition of Pectin from Acid Extracts of Whole Fruit after Removal of Electrolytes<sup>a</sup>

Extract of Whole	Aliquot of Original Solution Ml.	Pectin Deposited				Average Mg./ml
		Mg.	Mg.	Mg.	Mg.	
Grapefruit	2.5	14.2	14.1	14.1	...	5.65
	5.0	28.4	28.1	28.2	28.0	5.64
	10.0	57.8	57.1	58.3	57.7	5.77
Orange	5.0	13.8	14.4	13.7	13.8	2.78
	10.0	29.0	28.9	28.7	28.5	2.88
Apple	5.0	10.9	11.1	11.1	11.1	2.22
	10.0	21.4	21.4	21.8	22.0	2.17
	25.0	55.2	54.8	56.7	56.2	2.23

<sup>a</sup> Averages of triplicate analyses by method of Emmet and Carré (4) yielded 5.16 mg. of pectin per ml. of grapefruit extract, 2.57 mg. per ml. of orange extract, and 1.99 mg. per ml. of apple extract.



**Table V. Electrodeposition of Pectic Acid from Extracts of Whole Apple and Grapefruit after Acetone Purification, Hydrolysis, and Removal of Electrolytes<sup>a</sup>**

Extract of Whole	Aliquot of Original Solution Ml.	Pectin Acid Deposited—				Average Mg./ml.
		Mg.	Mg.	Mg.	Mg.	
Apple	10.0	10.7	10.6	10.7	10.7	1.07
	20.0	22.1	22.1	22.1	22.1	1.10
Grapefruit	5.0	10.6	10.4	10.7	10.7	2.12
	10.0	21.1	21.0	21.5	21.2	2.12

<sup>a</sup> Averages of triplicate analyses by method of Emmet and Carré (4) yielded 1.07 mg. of pectic acid per ml. of apple extract and 2.13 mg. of pectic acid per ml. of grapefruit extract.

#### ANALYSES OF EXTRACTS OF GRAPEFRUIT, ORANGES, AND APPLES

The flavedo was removed from oranges and grapefruit, leaving most of the albedo intact. The apples were not peeled. The following modification of the extraction procedure of Myers and Baker (6) was used:

The fruit was cut up and disintegrated in a Waring Blendor with the addition of sufficient water and hydrochloric acid to give a thin slurry of pH 1.7 to 2.0. The slurry was then heated in a water bath at 85° C. for 30 minutes and filtered through analytical grade Celite. The residual pulp was extracted and filtered twice more in the same manner. The clear extracts were combined and diluted, if necessary, to a pectin content of approximately 1 mg. per ml. Aliquots of 100 ml. were taken for analysis by the calcium pectate method of Emmet and Carré. Electrolytes were removed from other portions of the solution

by ion exchange and the pectin was determined by electro-deposition and by the calcium pectate method (Table IV).

Analyses by the proposed method gave results from 9 to 13 per cent higher than those obtained by the method of Emmet and Carré.

None of the extracts used in this study contained starch or dextrans, as indicated by the absence of any color formation with iodine solution. It is probable that such interference could be obviated, when necessary, by amylolytic treatment prior to deashing.

Electrodepositions of pectic acid were also made from deashed aliquots of fruit extracts which had been subjected to the same preliminary acetone purification and hydrolysis as used for calcium pectate determination. These results were in good agreement with those obtained by the method of Emmet and Carré (Table V).

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## Analysis of Petroleum Oil-Soluble Sodium Sulfonates by Adsorption

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An adsorption method for separating and estimating the components of oil-soap mixtures has been developed, which compares favorably with aqueous alcoholic extraction analyses in accuracy and precision. It is rapid and convenient and entirely free from emulsion difficulties.

IN THE manufacture of medicinal white oil by the treatment of petroleum stocks with fuming sulfuric acid, sulfonic acids are formed, some of which remain dissolved in the oil layer after it is separated from the sulfuric acid sludge. Upon neutralizing the "acid oil" layer with alkali, oil-soluble sodium sulfonates are produced. These "mahogany soaps" are extracted with aqueous alcohol and are further refined to produce commercially important emulsifiers.

Products of this type are marketed principally as a blend consisting of approximately equal amounts of oil and soap, contaminated with small amounts of inorganic matter. To control the ratio of oil to soap in these mixtures, it is necessary to estimate at least one of these components. This may be done by separating the sulfonates from the hydrocarbons by means of some physical process. Distillation is not suitable because of the relatively high boiling points of the hydrocarbons, coupled with the unstable character of the sulfonates at temperatures in the neighborhood of 130° C. Since neither of the components crystallizes readily from their mixtures or from solutions of their blends, crystallization cannot be used to separate them.

Because of the greater solubility of the soaps in a water-alcohol mixed solvent, unsaponifiable matter or oil is usually extracted

selectively with petroleum ether, from soap dissolved in 50 per cent aqueous alcohol solution. Most of the oil dissolves in the petroleum ether and the aqueous alcohol retains in solution the major portion of the soap. By a systematic process of multiple extractions, soap and oil mixtures can be separated completely if the proper conditions are chosen. Archibald and Baldeschwieler (2) have described a method of analyzing petroleum sulfonates by this type of extraction. Since some of the separations were found to be incomplete, after employing the standardized procedure, the authors have described a method of correcting the oil fraction for the soap contained in it.

The large number of extractions and manipulations ordinarily required by this method are time-consuming, and severe emulsion difficulties are encountered with some types of samples.

Selective adsorption can be used to separate mixtures composed of materials having widely different adsorption characteristics, just as selective solvent action is used to separate materials of different solubilities. The adsorption process is often advantageous because of its convenience, speed, and freedom from emulsion troubles. An adsorbent must be found which will adsorb sodium sulfonates, since oil is not easily adsorbed, and the proper solvent and displacing agent must be chosen. Simple adsorption, like simple extraction, is usually inadequate for sharp separation of two components. Fractionation of some kind must be employed to the best advantage to achieve quantitative results.

This paper presents a new adsorption method which has the advantages of speed, convenience, and freedom from emulsion troubles, and which has been found to be especially useful in this laboratory for plant control work.



## PRELIMINARY EXPERIMENTS

Percolation experiments showed that sodium sulfonates could be selectively adsorbed from naphtha solution, and that they could, in turn, be completely displaced from the adsorbent by methanol.

## APPARATUS AND MATERIALS

The percolation apparatus, shown in Figure 1, consisted of a 250-ml. separatory funnel, *A*, a 250-ml. extraction flask, *B*, and a percolation tube, *C*. This tube had a 1.5-cm. inside diameter and was 40 cm. long. A delivery tube 5 cm. long and 3 mm. in inside diameter was sealed to the bottom of the percolator. Into it a glass wool plug was packed for the purpose of supporting the adsorbent. Just before starting the percolation, fresh Attapulugus clay was packed into the percolator by tamping successive 5-cm. sections of clay tightly in place with a rod. The clay was 30- to 60-mesh size and had been calcined at 482° C. (900° F.). (Silica gel of 28- to 200-mesh, from the Davison Chemical Corporation, and Fisher adsorption alumina for chromatographic analyses, were tried as sodium sulfonate adsorbents, but were found to be less efficient than Attapulugus clay. The clay was supplied by the Attapulugus Clay Co., 260 South Broad St., Philadelphia, Penna. Approximately 35 grams were used for an analysis.)

The volatility of the petroleum naphtha solvent used in these experiments was sufficiently above that of the oil in the sample, so that the solvent could be completely separated from the oil by evaporation. Naphtha of 30° to 80° C. (86° to 176° F.) boiling range, distilled from a paraffin-base crude oil, and A.S.T.M. precipitation naphtha (1) of 50° to 130° C. (122° to 266° F.) boiling range, were found to be satisfactory.

## PROCEDURE

Approximately 2.0 grams of sample are accurately weighed into extraction flask *B*, and dissolved in 25 ml. of petroleum naphtha. The solution is transferred to separatory funnel *A*, which is then stoppered. The stem of the funnel is placed inside the open end of percolator *C*, so that it just touches the clay, as shown in Figure 1. Upon opening the stopcock the solution will percolate down through the clay, and the stoppered separatory funnel will act as an automatic feeding device. The percolate issuing from the bottom of the clay column is caught in the tared extraction flask.

As soon as the last drop of solution has entered the percolator, the stem and inside of the funnel are washed with petroleum naphtha. These washings are charged to the percolator. The top of the percolator is also washed clean of any remnants of sample. Then 100 ml. of naphtha are charged to the separatory funnel and percolated through the clay using the same automatic feeding arrangement described for the solution.

If the total naphtha percolate is clear, it is set aside for evaporation. If it is cloudy, indicating the presence of unadsorbed soap or salts, the combined percolate is run through a second percolator packed with fresh clay. A 100-ml. naphtha wash is used as before. The combined naphtha percolate in the tared extraction flask is very carefully evaporated on a steam bath. Oil is prevented from creeping to the outside wall of the flask by blowing a gentle stream of air into the flask during the evaporation.

While this operation is taking place, 100 ml. of absolute methyl alcohol are percolated through each of the clay columns used to adsorb the soaps. The resulting percolates are collected in a tared extraction flask, and the solvent is evaporated from the soaps in the same manner as was described for the naphtha.

When practically all of the naphtha has been evaporated from the oil fraction on the steam bath, the extraction flask is placed in an oven and kept at 100° to 105° C. for 15 minutes, then cooled and weighed. This process of heating in the oven for 15-minute periods, followed by weighing, is repeated until the loss in weight

is less than 0.01 gram. Usually, 15 to 30 minutes of heating are sufficient.

Similar treatment is applied to the soap fraction dissolved in methyl alcohol, except that an oven kept at 120° to 130° C. is used to save time. The weights of oil and soap are reported as per cent of sample taken.

## TEST OF THE METHOD

Two tests were applied to this method. For the first test, 40 grams of a refined petroleum sulfonate (sample A, Table II) were separated into an oil and a soap fraction by the method of Archibald and Baldeschwieler (2). Settling times of from 8 to 16 hours were permitted for emulsions of naphtha and aqueous isopropyl alcohol to separate sharply into two layers. The oil fraction, however, had an ash content of 0.14 per cent; so it was filtered through Attapulugus clay to produce a colorless oil which contained no ash, and was, therefore, free of soap. A series of mixtures of this oil and the soap fraction was prepared, and then analyzed by the adsorption procedure. Table I contains these analyses and the corresponding known blend values. Sample 5 consisted of the extracted sulfonates themselves, without the addition of any oil.

In the second test, a similar series of known mixtures was prepared by blending oil and soap fractions which had been separated from a group of samples by the adsorption method. The adsorption analyses of these known mixtures are also presented in Table I.

These data indicate that the adsorption procedure gives accurate analyses of oil-soap mixtures of widely varying composition. As shown by the analysis of sample 5, the sodium sulfonates obtained by aqueous isopropyl alcohol extraction contained only approximately 0.3 per cent of oil. The oil fractions obtained in these adsorption analyses were practically colorless and free of soap, as indicated by the absence of ash following their ignition.

## PRECISION AND COMPARISON WITH EXTRACTION METHOD

A group of five refined soap samples was analyzed in duplicate to test the precision of the adsorption procedure (Table II). These data, together with the results shown in Table I indicate satisfactory reproducibility for most purposes.

Table I. Analyses of Known Blends of White Oil and Sodium Sulfonates by the Adsorption Procedure

Sample No.	According to Blend		Composition of Sample Found by Analysis		Deviation	
	Soap %	Oil %	Soap %	Oil %	Soap %	Oil %
1	19.6	80.4	20.0	80.0	+0.4	-0.4
2	37.6	62.4	38.0	62.2	+0.4	-0.2
3	58.7	41.3	59.2	40.9	+0.5	-0.4
4	82.5	17.5	82.5	17.6	0.0	+0.1
5	100.0	0.0	100.0	0.3	0.0	+0.3
6	10.1	89.9	10.6	89.7	+0.5	-0.2
7	19.6	80.4	19.8	80.1	+0.2	-0.3
8	35.0	65.0	34.7	65.0	-0.3	0.0
9	50.0	50.0	49.7	49.9	-0.3	-0.1
10	65.0	35.0	64.2	35.1	-0.8	+0.1
11	78.7	21.3	78.2	21.8	-0.5	+0.5
12	89.4	10.6	88.7	11.2	-0.7	+0.6

Samples 1 to 5 were blended from components prepared by aqueous isopropyl alcohol extraction. Samples 6 to 12 were blended from components prepared by Attapulugus clay adsorption.

Table II. Duplicate Adsorption Analyses of Refined Soaps

Sample	Composition by Analysis		Deviation	
	Soap %	Oil %	Soap %	Oil %
A	46.4	53.5	0.1	0.3
	46.5	53.8		
B	50.6	46.2	1.0	0.2
	49.6	46.0		
C	50.4	47.0	0.4	0.3
	50.8	47.3		
D	51.2	49.0	0.1	0.3
	51.3	48.7		
	49.0	50.3		
	49.8	49.3	0.8	1.0



Table III. Comparison of Analyses of Refined Soaps by the Adsorption and Extraction Methods

Sample	Adsorption Method			Composition by Analysis Extraction Method			Deviation	
	Soap	Oil	Total	Soap	Oil	Total	Soap	Oil
	%	%	%	%	%	%	%	%
F	56.8	42.7	99.5	56.6	43.1	99.7	+0.2	-0.4
G	60.3	39.1	99.4	59.8	39.6	99.4	+0.5	-0.5
H	55.7	44.4	100.1	54.0	44.8	98.8	+1.7	-0.4
I	56.6	24.8	81.4	58.1	23.4	81.5	-1.5	+1.4
J	67.4	32.8	100.2	66.6	33.4	100.0	+0.8	-0.6

Table IV. Resin-Displacing Power of a Series of Trial Eluants

Trial Eluant	Weight of Material Displaced	Ratio of Material Displaced to Resin on Clay
	Grams	
Acetone	1.490	1.9
Ethyl acetate	0.788	1.0
Diethyl ether	0.786	1.0
Chloroform	0.652	0.8
Ethylene dichloride	0.612	0.8
Nitromethane	0.562	0.7
Carbon tetrachloride	0.136	0.2
Carbon disulfide	0.032	0.0

Five other refined soap samples were analyzed by both the extraction method of Archibald and Baldeschwieler (2) and the adsorption procedure and the results are compared in Table III. Samples F and G were from the same manufacturer, but samples H, I, and J came from three different sources. Sample I was found to contain 18.6 per cent of material which was volatile at 130° C.

The agreement between the two methods appears to be satisfactory. They are probably capable of giving equal accuracy and precision on samples of this type.

SOAPS CONTAINING RESINS

Soaps that are produced in the course of medicinal white oil manufacture, as described above, have been found to be practically free of resinous (oxygenated hydrocarbon) components. There are some products on the market, however, which do contain resinous materials. These samples, containing resins in addition to sodium sulfonates and oil, present a more difficult analytical problem for both the extraction and the adsorption methods, than the simpler case of soap and oil mixtures. Whereas the extraction method usually gives somewhat high results for oil on these samples, the adsorption method gives high results for soap, due to the adsorption of resins as well as sodium sulfonates on the clay.

Since there is a difference in molecular structure and polarity between resin and sodium sulfonate molecules, there should also be an appreciable difference in their adsorption affinities. If all the sulfonate molecules in a given sample are more strongly adsorbed by clay than all the resin molecules, the latter can be selectively displaced, by the proper eluant, from a solid on which both components are adsorbed. By a series of trial experiments, ethyl acetate was found to be a good resin eluant. Several publications (3, 4, 5) have been helpful in choosing eluants.

CHOOSING THE RESIN ELUANT

A sample of soap, prepared by treating a very naphthenic petroleum stock with concentrated sulfuric acid, followed by neutralization with sodium hydroxide, was separated into its chief components: oil, resins, and sulfonates. This was done by extracting the soap from the oily matter (oil-resin mixture) by the aqueous isopropyl alcohol extraction method (2), and then separating the resin from the oil by percolating a petroleum naphtha solution of these components through a column of Attapulugus clay. The oil-free resin was recovered from the clay by displacing it with absolute methyl alcohol.

A known test mixture consisting of 34.5 per cent oil, 33.1 per cent resin, and 32.4 per cent soap was prepared and then dissolved in petroleum naphtha, and 25 ml. of this solution, containing 2.359 grams of the test mixture, were percolated through a

prepared clay column following the method described in the adsorption procedure. After the clay was washed with 100 ml. of petroleum naphtha, 150 ml. of a trial resin eluant were percolated through the column. This percolate was collected separately, and the weight of material displaced was determined by the method described for soaps. The ratio of this weight to 0.781 gram, the amount of resin in the percolator charge, was calculated.

Table IV contains the data obtained in this manner for a series of organic liquids.

Impure solvents were percolated through an excess of Attapulugus clay to remove small amounts of water and other easily adsorbed impurities.

Acetone displaced practically all of the resin and the sulfonates. Ethyl acetate and diethyl ether displaced a quantity of material practically equal to the weight of resin adsorbed. Since the displaced material contained negligible amounts of ash, it was assumed to be resinous matter. Ethyl acetate and diethyl ether were chosen as being the best resin eluants of the group tested.

MODIFIED ADSORPTION PROCEDURES

To check these conclusions, and to test a modified adsorption procedure for soaps of this type, a group of mixtures containing known amounts of oil, resin, and sulfonates was analyzed. The procedure employed was the same as the one previously described, except that after the 100-ml. petroleum naphtha wash was percolated through the clay, 100 ml. of the resin eluant were percolated in the same manner. The methyl alcohol percolation followed that of the resin eluant. The results of these analyses are shown in Table V.

Table V. Analyses of Known Blends of Oil, Resin, and Sodium Sulfonates by a Modified Adsorption Procedure

Sample No.	Composition of Known			Composition by Analysis			Resin Eluant
	Oil	Resin	Soap	Oil	Resin	Soap	
	%	%	%	%	%	%	
1	34.6	33.0	32.4	34.0	26.4	41.3	Chloroform
1	34.6	33.0	32.4	34.8	32.0	33.0	Diethyl ether
1	34.6	33.0	32.4	34.2	60.3	8.8	Acetone
2	55.0	5.0	40.0	57.3	5.7	38.4	Diethyl ether
3	49.8	15.5	34.7	50.5	14.5	34.5	Ethyl acetate
4	59.2	10.9	29.9	60.5	11.1	29.5	Diethyl ether

Table VI. Comparison of Analyses of Soaps Containing Resins by the Extraction and Adsorption Methods

Sample	Extraction Method			Adsorption Method			Deviation	
	Soap	Oily matter	Total	Soap	Oily matter	Total	Soap	Oily matter
	%	%	%	%	%	%	%	%
1	9.9	89.7	99.6	9.8	90.0	99.8	-0.1	+0.3
2	20.1	80.3	100.4	19.9	79.2	99.1	-0.2	-1.1
3	30.5	70.5	101.0	29.7	69.7	99.4	-0.8	-0.8
4	37.1	63.4	100.5	36.8	63.1	99.9	-0.3	-0.3
5	42.9	57.4	100.3	42.5	57.9	100.4	-0.4	+0.5
6	49.8	51.4	101.2	50.2	49.8	100.0	+0.4	-1.6

Diethyl ether and ethyl acetate were again found to be the best resin eluants, and the modified adsorption procedure using either of these liquids gave analyses of satisfactory accuracy.

In the analysis of petroleum sulfonates containing resins, it is usually sufficient to determine the sodium sulfonate content and the total amount of oily or inactive matter in the sample. The aqueous isopropyl alcohol extraction method (2) accomplishes this on samples of this type, but it is time-consuming. The adsorption method can also be applied to obtain only the soap and oily matter in these samples by simply replacing the petroleum naphtha, in the original procedure, with a suitable resin eluant. The first percolate will then contain both the oil and the resins and the methyl alcohol percolate will again contain the sulfonates.

A series of soaps prepared from a very naphthenic stock, as described above, and containing varying amounts of oil and resins, was analyzed by the isopropyl alcohol extraction method (2) and



also by the modified adsorption method. Ethyl acetate was used as the solvent for the oily matter, and methyl alcohol as the eluant for the sodium sulfonates. A comparison of the analyses is given in Table VI. Both methods gave essentially the same analyses, but the adsorption procedure was more rapid and convenient.

A known blend containing 49.8 per cent oil, 15.5 per cent resin (65.3 per cent oily matter), and 34.7 per cent sulfonates was analyzed by the ethyl acetate-methyl alcohol adsorption procedure; 66.2 per cent oily matter and 34.0 per cent soap was obtained.

In the case of petroleum sulfonates which are not known to be free of resins, it is therefore advisable to introduce an ethyl acetate percolation at the end of the petroleum naphtha wash, and before the methyl alcohol percolation, to test for the presence of resins. If the ethyl acetate displaces material which leaves practically no ash after ignition, the presence of resins is indicated, and one of the modified adsorption procedures described above should be adopted.

#### DISCUSSION

The chief advantages of the adsorption procedure are freedom from emulsion difficulties, rapid convenient physical operations, and sharp separations of oil and sodium sulfonate components.

Experience in this laboratory has demonstrated that a given adsorption procedure may fail to give correct analyses on different types of samples. The procedure used will depend upon the adsorption characteristics of the components in the refined oil-soluble sodium soap. Unless the product to be analyzed is known to consist entirely of oil and sodium sulfonates, as is normally the case, the mahogany soaps which are extracted by aque-

ous alcohol from caustic neutralized acid oil in the manufacture of medicinal white oil, it will be necessary to introduce an ethyl acetate percolation as a test for the presence of resins.

The adsorption procedure can be applied to crude oil-soluble sodium sulfonate products containing appreciable amounts of water and inorganic salts, after first removing these components. This removal can be accomplished very conveniently in the determination of salt and water by the usual methods. For salt, a precipitation-type method such as A.S.T.M. Method D91-40 (1) may be used, and for water a modified Dean and Stark method is very convenient.

Adsorption methods should find applications in grease and asphalt analyses, and in the separation of mixtures containing organic salts or acids and hydrocarbons.

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# Quantitative Determination of *d*-Galactose by Selective Fermentation

## With Special Reference to Plant Mucilages

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A simple method has been developed which permits the determination of small amounts of *d*-galactose in the presence of mannose, glucose, fructose, xylose, arabinose, and glucuronic acid with an accuracy of 92 to 98 per cent. It depends on differential fermentations with two yeasts, *Saccharomyces carlsbergensis* (N.R.R.L. No. 379) which ferments galactose, and *S. bayanus* (N.R.R.L. No. 966) which leaves galactose unfermented. The yeasts have little action on xylose, arabinose, or glucuronic acid, and these

compounds do not interfere with the determination. Reducing values of galactose, mannose, and *d*-glucurone were determined using the Munson-Walker method of analysis. The fermentation techniques were successfully applied to the hydrolysis products of lactose and to certain plant mucilages. The possible application of the method to galactose in the presence of galacturonic acid is being studied with a view toward its use in the analysis of other natural products.

RECENTLY, interest in the determination of galactose has been revived because of the newer technological applications of certain mannogalactan mucilages. The estimation of *d*-galactose, which thus assumes a new importance, has always presented difficulties, especially when other carbohydrates were present in quantity. The van der Haar modification of the Kent-Tollens-Creydt method (4), which depends on the oxidation of galactose to mucic acid, is not quantitative. Only when rigorous precautions are taken, and when relatively large amounts of galactose are present, does the procedure give fairly accurate results (14).

Ever since the earlier investigations of Kluyver (7), the quantitative estimation of *d*-galactose by fermentation with certain yeasts has interested chemists and microbiologists. Kluyver

found that galactose was fermented by two varieties of *Saccharomyces cerevisiae* and by a "milk sugar yeast". He also showed that *Schizosaccharomyces pombe*, *Torula monosa*, and *T. dattila* did not ferment galactose. On the basis of these differences, he proposed a proximate method for the microbiological determination of galactose in the presence of other sugars. Among those who used (and modified) Kluyver's procedures were Schmidt, Trefz, and Schnegg (11), Hopkins, Peterson, and Fred (6), Sherrard and Blanco (13), Kurth and Ritter (9), Kurth (8), Scott and West (12), Harding, Nicholson, and Grant (5), and very recently, Menzinsky (10). The last author showed that the strain of *Saccharomyces cerevisiae* which he used in galactose fermentation required preconditioning by culturing the yeast on



galactose-containing medium. Without such pretreatment the yeast was unable to utilize galactose.

Besides the yeasts mentioned above, the following are known to ferment galactose: *Saccharomyces pastorianus* (3), *S. marxianus* (3, 6), and *S. fragilis* (10). Other yeasts known to ferment the common hexoses other than galactose are *S. productivus* (3), *S. apiculatus* (3), and "Honey B" yeast (6). These lists are not exhaustive.

Kluyver used the evolution of carbon dioxide as a measure of fermentable sugars. Later investigators (6, 8) showed that a quantitative measure of the reducing value simplified the analysis of fermented sugar solutions.

Notwithstanding the extensive work on the selective fermentation of galactose, the results of relatively few experiments with pure sugar mixtures have been published and no attempt has been made to determine whether the methods could be applied in the presence of uronic acids. The limitations and general applicability of the fermentation methods are, therefore, indeterminate. The present study shows the usefulness of the microbiological method.

EXPERIMENTAL

The objects of the present investigation were to examine, more critically than heretofore, the application of differential fermentations to known sugar mixtures, noting their limitations, and to develop a proximate biochemical method for the determination of galactose in the hydrolyzates obtained from mucilages and hemicelluloses.

Several strains of *S. cerevisiae*, cultured in the laboratories of The Institute of Paper Chemistry, had been shown to ferment galactose quantitatively in 1937 by Kurth (8). In 1942, however, qualitative experiments with these same strains showed

that none fermented 1 per cent galactose solutions completely within 190 hours. It is evident that strains of *S. cerevisiae* may lose their potency as galactose fermenters with time, unless special precautions are taken to recondition them.

*Torula dattila* appeared at first to give fairly promising results as a nonfermenter of galactose. About 92 to 95 per cent of the galactose (in a 1 per cent solution), when treated with *Torula dattila*, could be recovered after 2 days at 30° C., but only about 80 per cent remained after a 5-day fermentation period. Whenever the concentrations of galactose dropped to 0.1 per cent, the sugar was rapidly destroyed.

The use of these organisms was discontinued in favor of two interesting yeasts obtained from L. J. Wickerham, Northern Regional Research Laboratory, Peoria, Ill. These were *Saccharomyces carlsbergensis* var. *mandshuricus*, N.R.R.L. No. 379 (originally obtained from Charles N. Frey of the Fleischmann Laboratories in 1940), a highly fermentative strain acting on dextrose, galactose, and some of the common disaccharides, and *S. bayanus*, N.R.R.L. No. 966 (also originally obtained from Frey in 1940), which was known to ferment dextrose, sucrose, and maltose, but which, qualitatively at least, had no effect on galactose. Neither yeast had (during the past three years) been kept on galactose media.

These organisms proved entirely satisfactory. Neither had more than a slight effect on arabinose, xylose, and glucuronic acid. No. 379 fermented *d*-glucose, mannose, fructose, and galactose almost quantitatively within 48 hours. No. 966 fermented the first three readily within the same time period and showed no action whatsoever on galactose. These differences led to the development of a satisfactory proximate method based on differential fermentations, in which the Munson-Walker reduction method (2) was used without modification. In aliquot portions, taken from fermentation mixtures, 20 to 125 mg. of galactose could be determined with an accuracy of 92 to 98 per cent, even when other sugars were present originally in great excess.

The yeasts were maintained in good condition by monthly transfers on glucose agar (Bacto-Dextrose agar, dehydrated). They have shown no decrease in potency over a period of 8 months. Agar slant cultures, 2 to 7 days old, were used for the preparation of the suspensions required in inoculating the sugar solutions. The following procedure was the same for either yeast.

About 2 ml. of sterile water were pipetted into the tube containing the agar slant culture, and the surface growth was removed gently by means of the pipet, which also served to stir the suspension briskly. For each bottle slant of glucose agar required, 0.5 ml. of the suspension was removed and spread over the surface by tilting the bottle back and forth. Eight-ounce, narrow-mouth, square, flint glass bottles with molded screw caps were used as containers for sterile water, yeast suspensions, and bottle slants. Thereupon, the slants were incubated for about 48 hours at 30° C. One bottle slant easily furnished enough inoculum for four subsequent sugar analyses, because a dense growth of yeast cells coated the agar surface at the end of the 48-hour period.

About 10 ml. of sterile water were added to the bottle slant, and the bottle was tilted back and forth to loosen the growth. About 5 ml. of the dense yeast suspension were pipetted into a sterile dilution bottle, and 20 to 30 ml. of sterile water were added. The exact amount of water depended upon the turbidity shown by a Cenco-Sheard-Sanford photometer. Suspensions whose readings fell within the range of 10 to 16 on this instrument, when distilled water gave a reading of 90, subsequently fermented sugar mixtures satisfactorily. If the initial photometer reading fell below 10, more water was added and thoroughly mixed with the suspension. Whenever the photometer reading exceeded 16, more yeast suspension was added. In practice it was found better to work with too dense than with too light a suspension. The density range listed above was shown by plate counts to approximate 35,000,000 cells per ml. This density also corresponded roughly to that of barium sulfate suspension prepared by mixing 5 ml. of a stock solution of 10 grams of barium chloride dihydrate per liter of water with 55 ml. of 1 per cent sulfuric acid and allowing the mixture to stand at least a week in a sealed con-

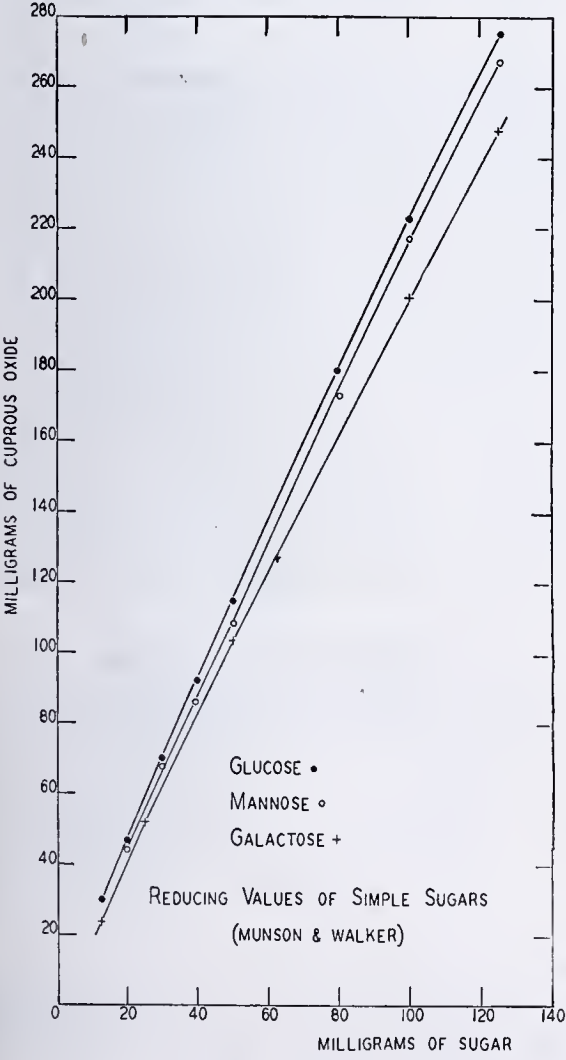


Figure 1



Table I. Action of Yeasts on Pentoses after a 6-Day Incubation Period

Pentoses Present in Aliquot Mg.	Treatment	Cu <sub>2</sub> O Obtained in Munson-Walker Determinations Mg.
12.5 (xylose)	Control	26.3
12.5 (xylose)	Organism 379	20.8
12.5 (xylose)	Organism 966	22.3
12.5 (arabinose)	Control	28.6
12.5 (arabinose)	Organism 379	26.7
12.5 (arabinose)	Organism 966	26.7

tainer. It was found expedient to use 25 ml. of sugar solutions in the fermentations and to carry out all experiments in 125-ml. Erlenmeyer flasks. The total reducing sugar in such solutions never exceeded 2 per cent, and the galactose concentration was ordinarily kept within the range of 40 to 250 mg. per 25 ml. To the sugar solution were added 15 ml. of a filtered yeast extract. [Red Star starch-free yeast cake was mixed with sufficient distilled water to yield a 10 per cent suspension. This was heated for 1 hour in an Arnold sterilizer at about 100° C. and subsequently for 20 minutes at 15 pounds' pressure (at 121° C.). The cooled suspension was filtered several times through fluted filter paper using Cellite to clarify the solution. Ordinarily the yeast extract formed a slightly turbid solution. This was dispensed in 80-ml. portions into Erlenmeyer flasks, which were plugged with cotton and heated at 15 pounds' pressure for 20 minutes. The cooled flasks of sterile yeast extract can be stored for months without change in a refrigerator.]

The sugar and yeast extract mixtures, which showed a pH of about 5 to 6 (alkacid paper), were then sterilized at 15 pounds' pressure for 15 minutes, cooled to about 30° C., inoculated under aseptic conditions with 10 ml. of the appropriate yeast suspension, and incubated at 30° C. for a minimum of 48 hours.

The fermentations were always run concomitantly in pairs, under identical conditions, one flask being inoculated with organism 966 and the other with organism 379. Three or four times during the incubation period, the flask was rotated gently to bring the bottom yeasts into intimate contact with the sugar solution. At the end of the fermentation period, each solution was diluted to 100 ml. with distilled water, thoroughly mixed, and filtered through two No. 50 Whatman filter papers. Twenty-five to 50-ml. aliquot portions of the clear, pale yellow filtrates were taken for analysis by the usual Munson-Walker technique. (Care must be taken to digest the asbestos used in Gooch crucibles thoroughly with hot Fehling solution, and with concentrated nitric acid. To prevent later clogging of Gooch crucibles, such treatments should be continued until asbestos filter pads permit the rapid filtration of hot Fehling solution containing the filtered yeast extract referred to above.)

The weight of cuprous oxide resulting from the fermentation with organism 379 was subtracted from that obtained by the use of organism 966. The galactose equivalent was calculated by the use of the galactose-cuprous oxide graph (Figure 1) drawn from data obtained experimentally with pure galactose. (The mannose values given in Figure 1 were obtained from pure *d*-mannose; the glucose values were taken from Munson and Walker's tables.)

The effects of *S. carlsbergensis* and *S. bayanus* on small amounts of xylose and arabinose were shown to be relatively unimportant, even after an incubation period of 144 hours instead of the usual 48-hour period. This is indicated in Table I (and Table II).

Inasmuch as the net reducing values in all differential galactose determinations were obtained by subtracting the weight of cuprous oxide found after fermentation with organism 379 from that found after a fermentation with organism 966, the over-all error resulting from the presence of pentoses is negligible. Furthermore, the authors' quantitative fermentation periods seldom exceeded 2 days, which presumably would result in a lessened action on the pentoses.

Because glucuronic acid may be a minor component of hemicellulose hydrolysis, the effect of *d*-glucurone in the galactose analysis was determined. Figure 2 shows the reducing values in milligrams of cuprous oxide plotted against the weights of glucurone (melting point 174–5° C.) taken for analysis. Over a fairly wide range, this curve is practically coincident with that of glucose. The action of organisms 379 and 966 on glucurone

was found to be very slight and their over-all effect in differential fermentations of galactose was almost negligible. This is indicated in the last row of Table II.

Orientating experiments were also carried out with purified galacturonic acid, which remains virtually unattacked by either organism, even when only small amounts of the acid are present in the usual fermentation mixture. The reducing value (Munson-Walker) of 12.5 mg. of galacturonic acid was shown to be 23.0 mg. of cuprous oxide; when neutralized, sterilized, inoculated, and incubated in the usual manner, 20.7 mg. and 20.2 mg. of cuprous oxide were obtained with organisms 379 and 966. Here again the "error" is cancelled.

The value and limitations of the selective differential fermentations in the determination of galactose are clearly shown in Table II. The first and last columns of this table should be compared. Invariably, in the higher concentrations, galactose shows a slight but persistent residual reduction after fermentation with organism 379. Whether this is due to very small amounts of unfermented galactose or (what is more probable) to the slight reducing power of the products of the fermentation is not known. The error is never very appreciable, but it accounts for the fact that galactose recoveries are usually somewhat low. Organism 966 is without effect on galactose.

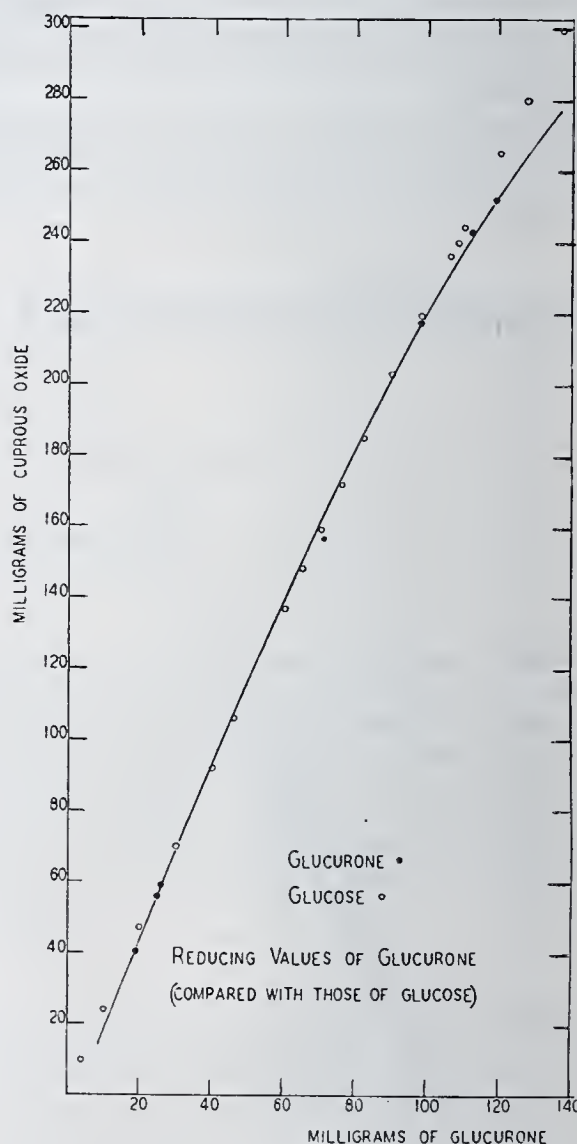


Figure 2

In order to determine whether galactose could be satisfactorily determined in the hydrolyzate of a disaccharide, pure lactose was heated with 2 per cent sulfuric acid at the boiling point for 2.75 hours. The solutions were nearly neutralized with solid sodium carbonate (pH about 5, alkacid test paper). Aliquot portions representing, in each case, 125 mg. of lactose (hydrate) were sub-



Table II. Galactose Determination Alone and in Mixtures of Pure Sugars

1 Galactose Taken	2 Other Compo- nents	3 Cu <sub>2</sub> O Obtained after Fermen- tation with No. 966	4 <sup>a</sup> Cu <sub>2</sub> O Obtained after Fermen- tation with No. 379	5 Net Weight of Cu <sub>2</sub> O, 3-4	6 Galactose Recovered (Calcd. from 5, by Use of Figure 1)
Mg.	Mg.	Mg.	Mg.	Mg.	Mg.
125	None	247.0	2.6	244.4	123.5
125	None	250.1	5.7	244.4	123.5
125	None	247.3	4.5	242.8	123.0
150	None	292.6	6.0	286.6	146.5
(Fermented 72 hrs.)					
None	Mannose 125	None	None	None	None
None	Mannose 125	Negligible <1	Negligible <1	None	None
62.5	Mannose 62.5	126.6	1.7	124.9	61.5
45	Mannose 80	91.2	1.6	89.6	42.5
25	Mannose 100	49.5	0.5	49	23.5
20	Mannose 230	37.1	None	37.1	18.5
40	{ Mannose 40 Glucose 40 Xylose 5	88	8.8 (due to xylose)	79.2	37.5
40	{ Mannose 20 Glucose 20 Xylose 45	173	92.0 <sup>b</sup> (due to xylose)	81.0	38.5
25	{ Fructose 25.0 Mannose 62.5	51.2	1.6	49.6	23.8
30	{ Fructose 30.0 Mannose 75.5	60.5	None	60.5	29.0
37.5	Glucurone 12.6	101.55	25.75 <sup>c</sup> (due to glucurone)	75.8	36.0

<sup>a</sup> Unless otherwise stated, figure in column 4 represents error due to incomplete fermentation of galactose or to presence of small amounts of reducing substances among galactose fermentation products.  
<sup>b</sup> Recovered (using Allihn's factor), 40.7 mg. of xylose.  
<sup>c</sup> Recovered, 13 mg. of glucurone.

ected to differential fermentation. The galactose values found were 62.5 and 62.5 mg. ( $\approx$  126.5 and 126.6 mg. of cuprous oxide); the calculated value is 62.5 mg. of galactose.

The effect of fructose on the galactose determination is negligible. Fructose, when present in large amount, shows a persistent reducing value after fermentation with either organism. Inasmuch as these copper values were practically identical for both No. 379 and No. 966, the errors cancelled each other. In a set of fermentations with fructose alone, 250 mg. of this sugar yielded 7.1 mg. of cuprous oxide after fermentation with No. 966, and 6.9 mg. of cuprous oxide after treatment with No. 379.

Acid hydrolyzates of polysaccharides (such as gums or hemicelluloses) are ordinarily neutralized with purified barium carbonate. To avoid the introduction of barium ion, which may or may not be deleterious to yeasts, sodium carbonate was used in lowering the acidity of the sugar solutions. These were never rendered alkaline. Alkacid test paper showed that the pH was about 5 to 6, which experience had shown to be satisfactory for yeast fermentations. Relatively large amounts of sodium sulfate had practically no effect on either the rate of fermentation of the sugar or on the results of the Munson-Walker determination.

The above analytical method was applied to a series of mannogalactan mucilages isolated from the endosperms of various seeds by extraction with hot water, filtration, and precipitation with ethanol. The dried mucilages were hydrolyzed by boiling for about 6 hours with 25 ml. of 2 per cent sulfuric acid. The solutions were cooled, brought to a pH of 5 to 6 with solid sodium carbonate, and subjected to the differential fermentation without removing the solution from the 125-ml. flask. The analyses were made as usual on 25- or 50-ml. filtered aliquots taken from the fermentation mixtures that had been diluted to 100 ml.

The sugar yields found in such hydrolyses should be considered minimal values. Significant amounts of mannose were lost on hydrolysis, but galactose appeared to be largely unaffected. This was shown

by control experiments with pure sugar solutions that had been treated with 2 per cent sulfuric acid for 6 hours. One hundred milligrams of mannose yielded 217 mg. of cuprous oxide before and 210 mg. of cuprous oxide after acid treatment. The mannose reversion products, however, had no effect on the galactose determination. The acid-treated mannose fermented completely without reducing value. Galactose appeared to be unaffected by the action of 2 per cent sulfuric acid; 100 mg. of galactose yielded 200.5 mg. of cuprous oxide before and 201.0 mg. of cuprous oxide after acid treatment.

Table III gives the yields of galactose (calculated as galactan on the oven-dry, ash-free basis) of several endosperm mucilages, some of which have distinct technological interest.

DISCUSSION

Assuming a modicum of microbiological control, the relative simplicity of the differential fermentation method for galactose has obvious advantages over the older mucic acid procedure. It requires less material for analysis, less attention on the part of the analyst, and is less subject to fluctuations with slight variations in technique. It also appears to be more accurate.

*S. carlsbergensis* (organism N.R.R.L. No. 379) requires no reconditioning to galactose, such as that required by certain strains of *S. cerevisiae*. Organism N.R.R.L. No. 379 ferments galactose readily, despite the fact that this sugar has not been used as a nutrient in its culture. In the authors' brief experience, *S. carlsbergensis* does not lose its potency on transfer. It was as active in fermenting galactose after 8 months of culture as it was on receipt from Peoria. *S. bayanus*, although a powerful hexose fermenter, leaves galactose practically untouched. This difference in behavior should make for an ideal combination of microorganisms. On the other hand, the galactose fermentation by No. 379 leads to products which have a slight reducing value (as shown in Table II). Although very slight, the error thus caused must be taken into account. The reduction is also subject to minor fluctuations. In general, it leads to galactose values that are slightly low.

The usefulness of the method in its application to certain gums and mucilages is manifest. In the case of the endosperm mucilages, independent determinations of mannose (1), coupled with the figures given in Table III, account for 94 to 97 per cent of the total hydrolyzates. The possible application of the galactose method to the hydrolysis products of pectins, in which galacturonic acid residues predominate, is under investigation. How-

Table III. Galactan Content of Plant Mucilages

Mucilage	Galactan, %	Mucilage	Galactan, %
Guar 1 ( <i>Cyamopsis tetragonolobus</i> )	37.8, 38.2 <sup>a</sup> 37.4, 37.6 <sup>a</sup>	Arabogalactan (from <i>W. Larchwood</i> )	79.4, 79.5 <sup>a</sup> 80.1
Guar 2	33.8, 34.4 <sup>a</sup>	Palo verde ( <i>Cercidium torreyanum</i> )	21.3, 21.9 <sup>a</sup>
Locust bean ( <i>Cerantonia siliqua</i> )	20.0 19.9	Tara ( <i>Caesalpinia spinosa</i> )	26.2, 26.4 <sup>a</sup>
Honey locust ( <i>Gleditsia triacanthos</i> )	26.0 25.9	Huizache ( <i>Caesalpinia cacaloca</i> )	27.7, 27.3 <sup>a</sup>
Flame tree ( <i>Delonix regia</i> )	18.2, 18.9 <sup>a</sup>	<i>Sophora japonica</i>	15.6
Kentucky coffee bean ( <i>Gymnocladus dioica</i> )	26.2, 26.8 <sup>a</sup> 25.4, 25.6 <sup>a</sup>		

<sup>a</sup> Duplicate determinations on aliquots from same fermentation are given on same line.



ever, a few preliminary experiments indicate that galacturonic acid is affected but little by either organism.

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# Use of the Discriminant Function in the Comparison of Proximate Coal Analyses

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Comparing a number of analyses of a material from one source with analyses of similar material from another source has heretofore presented a very real problem. The present paper applies statistical methods to the comparison of proximate analyses and B.t.u. per pound of coal from two mines. This statistical analysis by random sampling shows the probability that two coals come from the same source. The discriminant function developed by Fisher is used. By means of this function a comparison may be obtained between analytical data based on material from one source and data on similar material from a different source. In addition, the order of significance of the several analytical constituents in a single series of samples may be determined. The method is capable of general application.

FISHER (6), in 1936, developed the discriminant function for the comparison of multiple measurements obtained in taxonomic problems. Since then three papers (3, 4, 5) have appeared which make use of Fisher's technique. The present paper is concerned with the application of the discriminant function to the differentiation of two series of proximate coal analyses and the B.t.u. per pound of coal. Each series of analytical data is from a different mine.

Each series consists of 100 samples of coal. The proximate analysis, covering the volatile matter, fixed carbon, per cent of ash, as well as the B.t.u. per pound of coal, is used in making the comparison of the coal from these mines. The samples were taken from cars of coal sent to this college over a period of several years, and the analyses are reported on samples dried at 105° C. The analytical data, while accurate, indicate that the methods of sampling may be questioned. The value of the present approach is, however, not in the data reported but in the application of statistical procedures to comparison of similar chemical data. The discriminant function enables one to obtain a numerical comparison of the coals by the use of two linear compounds or equations in which the effects, in the present instance, of all four of these measurements are combined. Further, the application of this function to these analytical data permits a test for signifi-

cance between the constituents of which the compounds are formed.

The compound for the first mine is:

$$X = b_1x_1 + b_2x_2 + b_3x_3 + b_4x_4$$

where  $x_1$ ,  $x_2$ ,  $x_3$ , and  $x_4$  represent, respectively, B.t.u., per cent of volatile material, per cent of fixed carbon, and per cent of ash, and  $b_1$ ,  $b_2$ ,  $b_3$ , and  $b_4$  are constants to be found. The variables  $x_1$ ,  $x_2$ ,  $x_3$ , and  $x_4$  may be correlated. The compound for the second mine is

$$X' = b_1x'_1 + b_2x'_2 + b_3x'_3 + b_4x'_4$$

where  $x'_1$ ,  $x'_2$ ,  $x'_3$ , and  $x'_4$  represent, respectively, the above similar measurements; the coefficients are the same as in compound  $X$ . The difference between the means of the above two compounds made up of the four measurements is

$$D = b_1d_1 + b_2d_2 + b_3d_3 + b_4d_4 \quad (1)$$

where  $d_1 = \bar{x}_1 - \bar{x}'_1$ ,  $d_2 = \bar{x}_2 - \bar{x}'_2$ ,  $d_3 = \bar{x}_3 - \bar{x}'_3$ , and  $d_4 = \bar{x}_4 - \bar{x}'_4$ , and  $\bar{x}_1$ ,  $\bar{x}_2$ ,  $\bar{x}_3$ , and  $\bar{x}_4$  represent the arithmetic means of the respective measurements made of the coal from the first mine and  $\bar{x}'_1$ ,  $\bar{x}'_2$ ,  $\bar{x}'_3$ , and  $\bar{x}'_4$  represent the means of similar measurements made on the coal from the second mine.

Table I. Measurements of B.t.u., Per Cent of Volatile Material, Per Cent of Fixed Carbon, and Per Cent of Ash for Mines A and B

Mine A				Mine B			
B.t.u., $x_1$	Vola- tile matter, $x_2$	Fixed carbon, $x_3$	Per cent ash, $x_4$	B.t.u., $x'_1$	Vola- tile matter, $x'_2$	Fixed carbon, $x'_3$	Per cent ash, $x'_4$
13,000	25.7	64.3	7.9	13,600	33.0	59.6	6.5
13,700	25.0	64.2	8.4	14,300	36.9	56.3	6.2
12,800	23.0	66.2	10.0	13,000	35.5	50.9	12.9
12,300	22.8	59.9	12.9	14,000	34.9	58.7	5.8
14,100	33.5	60.2	5.9	13,700	30.1	59.3	10.0
...	...	...	...	...	...	...	...
13,900	27.3	59.4	8.3	13,800	35.2	54.8	9.5
Av. 13,110	28.33	61.16	8.71	13,596	34.00	56.28	8.0



Table I contains a few measurements from mines A and B, taken at random from the sets of observations. The means of the 100 measurements on each of four properties are given in the last line of that table.

Let

$$S = b_1^2 s_{11} + b_2^2 s_{22} + b_3^2 s_{33} + b_4^2 s_{44} + 2b_1 b_2 s_{12} + 2b_1 b_3 s_{13} + 2b_1 b_4 s_{14} + 2b_2 b_3 s_{23} + 2b_2 b_4 s_{24} + 2b_3 b_4 s_{34}$$

where

$$s_{ij} = \sum_{k=1}^{100} (x_{i,k} - \bar{x}_i)(x_{j,k} - \bar{x}_j) + \sum_{k=1}^{100} (x'_{i,k} - \bar{x}'_i)(x'_{j,k} - \bar{x}'_j)$$

where  $k$  is the variable of summation. The value of  $s_{ij}$  when  $i = 1$  and  $j = 1$  is

$$s_{11} = \sum_{k=1}^{100} (x_{1,k} - \bar{x}_1)^2 + \sum_{k=1}^{100} (x'_{1,k} - \bar{x}'_1)^2$$

In our case this quantity is (using values in Table I) found as follows:

$$s_{11} = (13,000 - 13,110)^2 + (13,700 - 13,110)^2 + \dots + (13,900 - 13,110)^2 + (13,600 - 13,596)^2 + (14,300 - 13,596)^2 + \dots + (13,800 - 13,596)^2 = 20,110,000 + 17,718,400 = 37,828,400$$

The value of  $s_{ij}$  where  $i = 2$  and  $j = 3$  is

$$s_{23} = \sum_{k=1}^{100} (x_{2,k} - \bar{x}_2)(x_{3,k} - \bar{x}_3) + \sum_{k=1}^{100} (x'_{2,k} - \bar{x}'_2)(x'_{3,k} - \bar{x}'_3)$$

By using the values in Table I this is

$$s_{23} = (25.7 - 28.33)(64.3 - 61.16) + (25.0 - 28.33)(64.2 - 61.16) + \dots + (27.3 - 28.33)(59.4 - 61.16) + (33.0 - 34.00)(59.6 - 56.28) + (36.9 - 34.00)(56.3 - 56.28) + \dots + (35.2 - 34.00)(54.8 - 56.28) = -645.82 - 373.49 = -1,019.31$$

The quantity  $S$  is equal to the sum of squares within compounds. By maximizing the quantity  $D^2/S$  the following equations arise. These equations are actually proportionalities, but for the purpose of evaluating constants  $b_1$ ,  $b_2$ ,  $b_3$ , and  $b_4$  they may be used.

$$s_{11}b_1 + s_{12}b_2 + s_{13}b_3 + s_{14}b_4 = d_1$$

$$s_{12}b_1 + s_{22}b_2 + s_{23}b_3 + s_{24}b_4 = d_2$$

$$s_{13}b_1 + s_{23}b_2 + s_{33}b_3 + s_{34}b_4 = d_3$$

$$s_{14}b_1 + s_{24}b_2 + s_{34}b_3 + s_{44}b_4 = d_4$$

From these equations the values of the  $b$ 's, of the coefficients in compound  $X$ , can be found. The solution of these equations gives, of all linear compounds in  $x_1$ ,  $x_2$ ,  $x_3$ , and  $x_4$ , the one compound,  $X$ , which most clearly discriminates one mine from the other. These equations are:

$$37,828,400.00b_1 + 123,906.00b_2 + 20,685.20b_3 - 140,927.00b_4 = 486.00$$

$$123,906.00b_1 + 1,614.87b_2 - 1,019.31b_3 - 571.21b_4 = 5.67$$

$$20,685.00b_1 - 1,019.31b_2 + 1,849.62b_3 - 849.97b_4 = -4.88$$

$$-140,927.00b_1 - 571.21b_2 - 849.97b_3 + 1,519.42b_4 = -0.06$$

There are several ways of solving these equations. One way (2), using the computing machines, is as follows: Dividing each equation by the absolute value of the coefficient of  $b_1$  in it gives:

$$(A) \quad b_1 + 0.003275b_2 + 0.000547b_3 - 0.003725b_4 = 0.000013$$

$$(B) \quad b_1 + 0.013033b_2 - 0.008226b_3 - 0.004610b_4 = 0.000046$$

$$(C) \quad b_1 - 0.049277b_2 + 0.089418b_3 - 0.041091b_4 = -0.000236$$

$$(D) \quad -b_1 - 0.004053b_2 - 0.006031b_3 + 0.010782b_4 = 0.000000$$

$$(A) - (B) \quad -0.009758b_2 + 0.008773b_3 + 0.000885b_4 = -0.000033$$

$$(B) - (C) \quad 0.062310b_2 - 0.097644b_3 + 0.036481b_4 = 0.000282$$

$$(C) + (D) \quad -0.053330b_2 + 0.083387b_3 - 0.030309b_4 = -0.000236$$

Dividing each equation by the absolute value of the coefficient of  $b_2$  in it gives:

$$(E) \quad -b_2 + 0.899057b_3 + 0.090695b_4 = -0.003382$$

$$(F) \quad b_2 - 1.567068b_3 + 0.585476b_4 = 0.004526$$

$$(G) \quad -b_2 + 1.563604b_3 - 0.568329b_4 = -0.004425$$

$$(E) + (F) \quad -0.668011b_3 + 0.676171b_4 = 0.001143$$

$$(F) + (G) \quad -0.003464b_3 + 0.01714b_4 = 0.000101$$

Dividing each equation by the absolute value of the coefficient of  $b_3$  in it gives:

$$(H) \quad -b_3 + 1.012215b_4 = 0.001711$$

$$(I) \quad -b_3 + 4.950058b_4 = 0.029157$$

$$(H) - (I) \quad -3.937843b_4 = -0.027446; \quad b_4 = 0.006970$$

$$\text{From (H)} \quad b_3 = 0.005344$$

$$\text{From (I)} \quad b_3 = 0.005345; \quad b_3 = 0.005345 \text{ (average)}$$

$$\text{From (E)} \quad b_2 = 0.008820$$

$$\text{From (F)} \quad b_2 = 0.008821$$

$$\text{From (G)} \quad b_2 = 0.008821; \quad b_2 = 0.008821 \text{ (average)}$$

$$\text{From (A)} \quad b_1 = 0.000007$$

$$\text{From (B)} \quad b_1 = 0.000007$$

$$\text{From (C)} \quad b_1 = 0.000007$$

$$\text{From (D)} \quad b_1 = 0.000007; \quad b_1 = 0.000007 \text{ (average)}$$

Therefore

$$b_1 = 0.000007, \quad b_2 = 0.008821, \quad b_3 = 0.005345, \quad b_4 = 0.006970$$

This method of solving simultaneous equations is easy to follow and easy to explain to the average computer.

The linear compound which enables one to detect the greatest difference between the mines in relation to these four measurements is

$$X = 0.000007x_1 + 0.008821x_2 + 0.005345x_3 + 0.006970x_4$$

The mean compound pertaining to mine A is

$$\bar{X} = 0.000007\bar{x}_1 + 0.008821\bar{x}_2 + 0.005345\bar{x}_3 + 0.006970\bar{x}_4 \\ = 0.000007(13,100) + 0.008821(28.33) + 0.005345(61.16) + 0.006970(8.71)$$

or

$$\bar{X} = 0.7293$$

The mean compound pertaining to mine B is

$$\bar{X}' = 0.000007(13,596) + 0.008821(34.00) + 0.005345(56.28) + 0.006970(8.65) = 0.7562$$

The difference  $D$  between these two means is  $0.7562 - 0.7293 = 0.0269$ . This can be found directly from Equation 1 as follows:

$$D = 0.000007(486) + 0.008821(5.67) + 0.005345(-4.88) + 0.006970(-0.06)$$

or

$$D = 0.003402 + 0.050015 - 0.026083 - 0.000418 = 0.0269 \quad (2)$$

as before.

The question arises as to whether or not the means of these compounds are statistically significantly different. Table II contains an analysis of variance of these compounds and enables



one to test for a significant difference between these two means. The two asterisks in the last column indicate that these compounds are highly significantly different. This means that the probability of getting by chance such a large value of  $D$  is less than 0.01. This is found from a table of  $F$  values (1, 7). The odds in favor of getting by chance such a large difference between the compounds pertaining to the two mines are less than 1 to 99. This indicates that the coal from mine A is definitely different from the coal from mine B, as far as these four measurements are concerned.

By examining the four terms in Equation 2 one can determine which characteristic of the coal (B.t.u., per cent of volatile material, per cent of fixed carbon, or per cent of ash) is most important for differentiating the two coals, which is of next importance, etc. The second term, 0.050015, in this equation is greater than the absolute value of each of the other terms; hence the per cent of volatile material is the most important factor, in this case, for determining whether or not the coal from one mine is different from the coal from the other mine. The absolute value of the third term in this equation, 0.026083, is second in size; hence the per cent of fixed carbon is next in importance for revealing a difference between the coal from the two mines. The factor B.t.u. is third in importance for differentiating these two coals. Per cent of ash is of the least importance for these mines. The order of importance of these factors may change for other coals. Table III gives the ranks of importance of these characteristics when various combinations are used to calculate the compounds.

Table III gives the ranks of the characteristics of the coals for various combinations of the sets of measurements. In column 2 it is seen that per cent of fixed carbon is the most important characteristic for differentiating one mine from the other as far as per cent of volatile material, per cent of fixed carbon, and per cent of ash are concerned.

Table II. Analysis of Variance of the Compounds

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square
Total	199		
Between compound means	4	$50D^2 = 0.0362$	0.00905**
Within	195	$D = 0.0269$	0.00014

Table III. Rank of Characteristics for Various Compounds Pertaining to Coals A and B

	Compound Composed of				
	B.t.u. Per cent of volatile matter	Per cent of volatile matter Per cent of fixed carbon Per cent of ash	B.t.u. Per cent of carbon Per cent of volatile matter	Per cent of carbon Per cent of ash	Per cent of volatile matter Per cent of fixed carbon
B.t.u.	3	..	2	..	..
Volatile matter	1	2	1	..	2
Fixed carbon	2	1	..	1	1
Per cent ash	..	3	..	2	..

Table IV contains the means of measurements pertaining to B.t.u., volatile material, fixed carbon, and per cent of ash for two other mines, C and D. The discriminant function pertaining to these mines for the value  $D$  is

$$D = 0.000020 (495) - 0.006360 (5.10) - 0.001559 (-2.63) - 0.000468 (-2.16)$$

$$\text{or } D = 0.00990 - 0.00350 + 0.00410 + 0.00101$$

$$D = 0.01151$$

An analysis of variance table (not given) shows that the two coals are significantly different. The characteristic B.t.u. is

Table IV. Arithmetic Averages of B.t.u., Per Cent of Volatile Material, Per Cent of Fixed Carbon, and Per Cent of Ash Measurements from Mines C and D

Mines	B.t.u.	Per Cent of Volatile Matter	Per Cent of Fixed Carbon	Per Cent of Ash
C	13,700	35.09	57.18	6.56
D	13,205	29.99	59.81	8.72
Difference	495	5.10	-2.63	-2.16

Table V. Averages of B.t.u., Per Cent of Volatile Material, Per Cent of Fixed Carbon, and Per Cent of Ash for Mines E and F

Mines	B.t.u.	Per Cent of Volatile Matter	Per Cent of Fixed Carbon	Per Cent of Ash
E	14,012	34.15	60.31	4.21
F	12,134	35.78	48.14	13.25
Difference	1,878	-1.63	12.17	-9.04

most important, per cent of fixed carbon is second, per cent of volatile material is third, and per cent of ash is last in importance in testing whether or not the mines differ as far as a compound of these four sets of measurements is concerned.

Table V contains averages of measurements pertaining to B.t.u., volatile material, fixed carbon, and per cent of ash for mines E and F.

The difference between the means of the two discriminant functions pertaining to the mines is

$$D = 0.000083 (1878) - 0.001356 (-1.63) + 0.006124 (12.17) + 0.009580 (-9.04) = 0.155874 + 0.002210 + 0.074529 - 0.086603 = 0.146100$$

In this case B.t.u. is first, per cent of ash is second, per cent of fixed carbon is third, and per cent of volatile material is fourth in importance for differentiating between the coal from these mines.

## DISCUSSION

The discriminant function enables one to test for a statistical difference between two linear compounds made up of several variables or measurements. It has many advantages because it furnishes one measurement pertaining to a combination of several measurements.

In the present instance the statistical analysis of the data shows a difference between the coal from the two mines. It may be useful in the future to compare analytical data from other mines. Such an effort would involve the selection of a standard coal, and it seems apparent that other analytical data, such as per cent of sulfur, per cent of moisture, and the fusing temperature of the ash, should be included in the more accurate statistical comparison.

## CONCLUSION

Fisher's discriminant function has been applied to the differentiation of analytical data obtained from one hundred coal samples taken from each of two mines. The intercomparison of the relative significance of the elements of the analytical data relating to coal has been accomplished. The probability that two coals come from the same source by random sampling is given.

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# Colorimetric Analysis of Xanthone Spray Residues

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A colorimetric method for the determination of xanthone spray residues consists in adding a measured quantity of toluene to a sample of apples or apple plugs in a glass jar and shaking for 5 minutes. The resulting solution of xanthone and apple waxes is filtered and an aliquot of the filtrate taken for analysis. The xanthone is reduced to xanthhydrol by refluxing with sodium amalgam in toluene and methanol. After removal of the methanol by a water extraction, an aliquot of the supernatant toluene solution is swirled in concentrated hydrochloric acid, effecting an equilibrium transfer of the xanthhydrol to the acid layer to give a yellow color, which is measured photometrically.

**X**ANTHONE has been used experimentally as an insecticide against codling moth larvae and other insects. The purpose of this study was to develop a satisfactory method for determining xanthone residues on sprayed apples, and the following colorimetric procedure is recommended. It is based on a procedure used by the Laurel Hill Laboratory of the General Chemical Company for determining xanthone spray residues. The writers are indebted to several of the staff of this laboratory for constructive criticism and advice in the preparation of this paper.

## ANALYTICAL PROCEDURE

**REAGENTS.** Toluene. Toluene may contain an impurity, probably a thiophene derivative, which upon shaking with concentrated hydrochloric acid yields a slight yellow color in the acid layer. This impurity can be removed by adding 50 ml. of concentrated sulfuric acid per liter of toluene, allowing to stand over 24 hours, separating the layers, and distilling the toluene. The first milky portion of the distillate is discarded. Toluene from all operations may be recovered and reused if treated in this manner. White crystals of *p*-toluenesulfonic acid may appear in the toluene layer during the sulfuric acid treatment, but they do not distill and do not interfere. c.p. toluene usually does not contain this impurity.

Methanol (absolute).

Sodium amalgam. Cautiously melt 9 grams of sodium in 20 ml. of toluene in a round-bottomed flask and add 750 grams of mercury, drop by drop at first and more rapidly after a few milliliters have been added. Most of the toluene will volatilize, but some should be kept over the amalgam when it is transferred to an airtight bottle.

Hydrochloric acid (c.p. concentrated).

**PREPARATION OF STANDARDS.** Carefully weigh 50.0 mg. of pure xanthone, transfer to a 250-ml. volumetric flask, and make to volume with toluene. Keep tightly stoppered to prevent loss of toluene. One milliliter of this solution contains 200 micrograms of xanthone. If pure xanthone is not available for standards, it can be prepared by distilling a crude xanthone product and recrystallizing the distilled material several times from dioxane or other suitable solvent to a constant melting point of 174° C. Measure 2 ml. of the standard solution and sufficient toluene to make a total of 20 ml. into a 125-ml. flask fitted with a ground-glass joint. Add 10 ml. of methanol and 0.5 to 1.0 ml. of sodium amalgam, connect with a water-cooled condenser, and reflux for 30 minutes. Before removing the flask from the condenser, cool to prevent loss of toluene. Add 20 ml. of water and shake vigorously to remove the methanol from the toluene. Pour into a tall tube, such as a 50-ml. Nessler tube, retaining the amalgam in the flask. Allow the layers to separate, and pipet 5 ml. of the toluene layer (containing the xanthhydrol) into a 100- to 150-ml. flask that contains exactly 10 ml. of concentrated hydrochloric acid. Develop the color by swirling the mixture gently for approximately 1 minute. Pour into a test tube or cell and measure the color of the acid layer in a photometer. A glass color filter having maximum transmission at 424 millimicrons gave satisfactory results in a Type F Aminco photometer.

Repeat for 5- and 10-ml. aliquots of standard xanthone solution. The 5/20 aliquots of toluene used for color development

represent 100, 250, and 500 micrograms of xanthone. Prepare a standard graph by plotting the quantities of xanthone in the standard solution against the logarithms of the corresponding photometer readings. The light transmission of 148 micrograms of xanthone read under the conditions above in a 2.5-cm. (1-inch) cell is 50 per cent.

**ANALYSIS OF SAMPLES.** A statistical analysis has shown that 20 to 25 apples, taken from different parts of a tree, constitute a satisfactory sample for residue determination. Place the apples in a tared glass jar, weigh, and calculate the surface area from the weight, by the use of a previously established relationship. Add from 100 to 250 ml. of toluene, depending on the quantity of xanthone and the size of the apples, and shake for 5 minutes in a machine by the process described by Fahey *et al.* (1). Filter a portion of the solution, and use an aliquot not to exceed 10 ml. of the filtrate for the analysis as described under the procedure for standards. Read the amount of xanthone per aliquot from the standard graph.

Apple plugs may be treated in a similar manner. If they are used, a smaller volume of toluene can be used for stripping.

## DISCUSSION

**SOLVENTS FOR XANTHONE.** In selecting a solvent for the removal of xanthone residues, consideration was given to solubility, efficacy of wax removal from apples, and the extraction of interfering substances. Acetone, alcohols, benzene, and petroleum ether were not satisfactory because of poor solvent power for xanthone and too great solvent power for interfering substances.

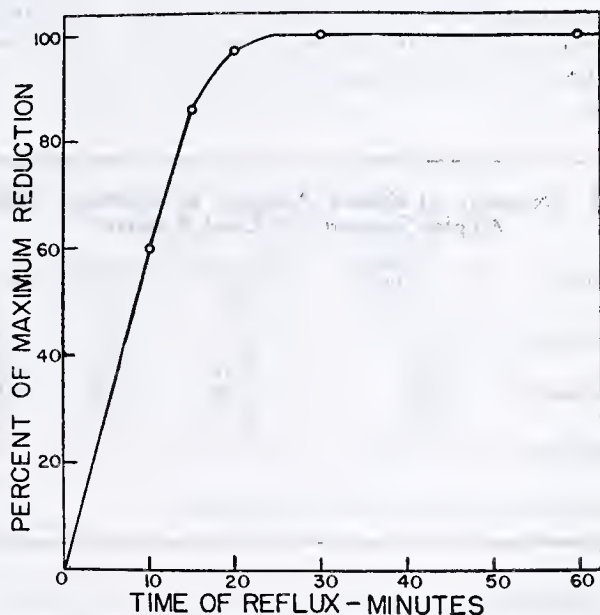


Figure 1. Rate of Xanthone Reduction to Xanthhydrol in Toluene-Methanol Mixture

The solubility of xanthone in toluene is 1.43 gram per 100 ml. at 30° C., which is far in excess of any concentration normally encountered in residue analysis. Since the apple wax appears to be completely dissolved, any xanthone that may be covered with wax is also obtained. The amount of interference introduced by shaking even mature waxy apples with toluene for 30 minutes is negligible. On the other hand, 5 minutes' shaking is sufficient for complete removal of xanthone residues. Toluene was therefore selected as the most desirable solvent for this method.

**REDUCTION OF XANTHONE.** It is best to use an aliquot of the residue solution containing from 0.4 to 2.0 mg. of xanthone for reduction. If the aliquot contains larger quantities, up to 50 mg., reduction will be complete, but further aliquoting and dilu-



tion after removal of the methanol will be necessary. The term "complete reduction" as used here means that, under the conditions of the method, a more intense yellow color cannot be obtained with a given amount of xanthone even if the time of reduction is doubled. Reduction of xanthone in a mixture of 20 ml. of toluene and 10 ml. of methanol reaches a maximum, under the reflux condition of the method, in 25 minutes (Figure 1), as judged by color development.

**COLOR DEVELOPMENT.** After reduction the addition of water followed by vigorous shaking removes the methanol from the toluene layer, which returns to its original volume of 20 ml. When the mixture is transferred to a container for the separation of the two layers, it is convenient to withdraw the amalgam for subsequent recovery. The 5-ml. aliquot of the toluene layer needed for color development can be taken before all the toluene has separated; it is not even necessary to filter to remove slight water turbidity. If smaller aliquots are used, sufficient pure toluene to make 5 ml. must be added before treatment with acid.

When xanthidrol is treated with hydrochloric acid, chlorine is substituted for the hydroxyl group. The resulting compound is colorless in dilute acid, but in the presence of concentrated hydrochloric acid an intense yellow color is produced. The intensity of yellow color is proportional to the quantity of xanthone used in the determination. If the toluene is removed from the acid layer to prevent a shift in the distribution ratio, the solution can be diluted with concentrated hydrochloric acid and the color still conforms to Beer's law.

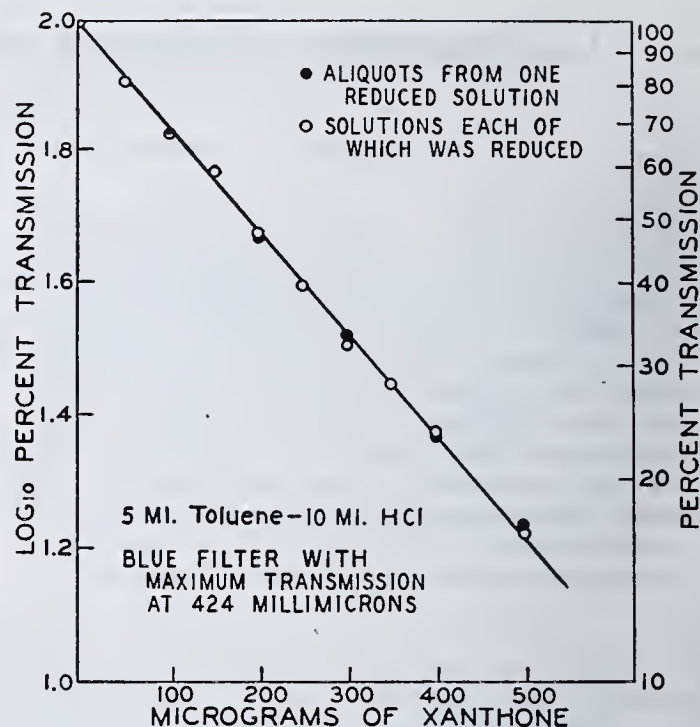
No decomposition of xanthidrol has been observed in toluene up to 12 hours after reduction, but low recoveries were obtained on some samples when the toluene layer was allowed to stand for a longer time. It is possible that the observed decomposition is due partly to oxidation of xanthidrol to xanthone, because a renewed reduction increases the yellow color upon subsequent acid treatment, but not to its original intensity. Standard solutions made directly from xanthone and toluene are stable for at least 60 days.

**Table I. Recovery of Known Amounts of Xanthone Added to Apples Sprayed with Lead Arsenate<sup>a</sup>**

Variety	Weight of Apples Grams	Xanthone Added Mg.	Xanthone Recovered Mg.	Recovery %
Jonathan apples	467	10.0	9.40	94.
	467	10.0	10.0	100.
	586	25.0	25.5	102.
Winesap apples	453	2.00	2.00	100.
	524	5.00	5.00	100.
	323	50.0	49.6	99.
Winesap plugs	142 sq. cm.	5.00	4.90	98.
			Av.	99.0

<sup>a</sup> Each sample contained 25 apples or 80 plugs.

**XANTHYDROL DISTRIBUTION RATIOS.** In this method there are two distributions of xanthidrol, between toluene and water-methanol solution and between toluene and hydrochloric acid. Both distribution ratios have been found to be constant for any total amount of xanthidrol up to 50 mg. for the former and 0.6 mg. for the latter, when the volumes are kept as specified in the method. The distribution ratio between toluene and hydrochloric acid was not studied beyond 0.6 mg., because the intensity of color at the corresponding concentration was more than sufficient for the method. Two per cent of the xanthidrol remains in the water-methanol solution, and 77 per cent of the total is transferred to the hydrochloric acid. The concentration of the hydrochloric acid is not too critical, since experience has shown no color differences in the range of 34 to 36.5 per cent acid, and whereas the use of lower strength acid will lead to lesser color intensities, constant results will be obtained if the standards are treated with the same acid. If conditions require the use of volumes other than those specified, a new standardization curve



**Figure 2. Representative Standard Xanthone Graph**

must be established. Since distribution ratios are involved in the method, it is imperative that all volumes be measured accurately.

When an aliquot is taken from the supernatant toluene layer, there is some tendency for redistribution of the xanthidrol upon standing 2 hours or longer. This effect is the more pronounced the larger the concentration of xanthidrol. No explanation can be given for this change in distribution, but it is constant for any constant volume ratio. If the procedure is followed as described, good results are obtainable. If the toluene solution is to be retained for checking color development on the same day, it should be separated from the water-methanol solution before an aliquot is removed.

The distribution of xanthidrol between toluene and hydrochloric acid rapidly comes to equilibrium, but as a check one should make duplicate determinations at this point in the procedure. After color development no change in intensity occurs, even after 24 hours, if precautions are taken with respect to apple-wax concentration in the toluene as described in the next section.

**INTERFERENCES.** Apples sprayed with lead arsenate alone and stripped for 5 minutes in toluene gave a photometer reading corresponding to that given with 0.1 microgram of xanthone per square centimeter. Similar samples stripped for 30 minutes did not show an interference greater than 0.15 microgram per square centimeter. When this type of interference was tested on Delicious, Rome, and Winesap varieties, no significant differences were found. The blank on apple plugs is also negligible. Xanthone deposits of 22 micrograms per square centimeter dropped to 1.6 micrograms after weathering for 4 weeks; therefore, if any decomposition products are formed, they either do not remain on the apple or do not cause any detectable interference with the method.

Apple wax does not interfere in the reduction of xanthone. It also causes no interference with the color developed in hydrochloric acid when the aliquot of strip solution is 10 ml. or less. It is possible to use a 15-ml. aliquot or even 20-ml. with early-season apples, but if turbidity occurs in the acid phase, the wax concentration in toluene must be reduced in some way. The yellow color developed in hydrochloric acid is stable for 24 hours or more, but should be read within an hour, since turbidity may develop on long standing. This effect is most marked when the



toluene is in contact with the acid layer overnight and is probably due to temperature changes.

Ground-glass joints are preferred for the reflux reduction. Rubber stoppers may be used if they are boiled in normal alkali for 15 minutes, then in normal sulfuric acid for 15 minutes longer, and finally rinsed well with distilled water.

ACCURACY AND PRECISION. One standard graph obtained in this investigation is given in Figure 2. The absorption cells used were standard test tubes (2-cm. inside diameter). The line was fitted by the method of least squares. The standard error of estimate for the points on this line is  $\pm 6.7$  micrograms. Aliquots can be so chosen as to permit readings on 200 to 500 micrograms of xanthone, thus allowing reduction of the percentage standard error to 3.3 or less. More accurate and precise results can be obtained if the colored solutions are read in cells with flat optical windows, since the test tubes used in this work vary about 0.5 per cent in light transmission.

Recovery experiments were run by adding known amounts of xanthone to apples that had been sprayed with lead arsenate, and analyzing by the described procedure (Table I). The average recovery from seven samples was 99.0 per cent, which is not statistically different from complete recovery.

The completeness of removal of xanthone from Winesap and Rome apples (collected 6 weeks before harvest) was also studied by submitting samples to a second stripping with toluene. The amount of xanthone removed by the second treatment was calculated after allowing for the amount of toluene left on the apples from the first stripping. Six samples, having from 6 to 13 mg. of xanthone per sample, treated in this manner, showed a removal of 99 per cent or better with the first 5-minute stripping treatment.

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PHENOL STUDIES  
Qualitative Tests for Phenol and o-, m-, and p-Cresol

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Qualitative tests for phenol employing ferric chloride, hypochlorite, or the reagents of Melzer, Millon, Liebermann, Guareschi, and Cotton have been modified to permit differentiation between phenol and o-, m-, and p-cresol.

THE manufacture and use of phenol and cresol on a large scale have furnished an incentive for investigating the toxicity and metabolism of these compounds, and these investigations, in turn, have called for a review of analytical methods useful in their detection and estimation. A review of quantitative methods for the estimation of phenol in biological material, including a spectrophotometric procedure for the quantitative estimation of free, conjugated, and total phenol in tissues and fluids, has been published (2, 3). In this paper qualitative tests for o-, m-, and p-cresol which are modifications of well known qualitative tests for phenol are described. The hypochlorite test for o-cresol offered here has apparently not been recorded before.

A single test or a combination of several of these color tests can be used very effectively for the identification of phenol or o-, m-, or p-cresol, if the unknown solution contains only one of these compounds. If the unknown contains two or more of these substances, positive identification of each compound is not always possible; m-cresol, when present in mixtures in a low concentration, is particularly apt to escape recognition.

In analyzing for these compounds, even though each substance gives very similar color reactions in high and in low concentrations, it is best to prepare and test dilutions that approach the ranges of sensitivity. These concentrations will furnish in addition some rough idea of the quantities present.

The phenol used was Merck's reagent quality, and the o-, m-, and p-cresol, obtained from the Eastman Kodak Company, was believed to be from 96 to 98 per cent pure. The melting points of these cresols are 30-31°, 10-11°, and 32-34° C., respectively.

QUALITATIVE TESTS

DETECTION OF PHENOL AND DERIVATIVES CONTAINING PHENOL-HYDROXY GROUPING. The sensitivity of Millon's test (7) depends to some extent upon the quantity of mercury

used and the manner of preparing the reagent. For these studies the latter was prepared by dissolving 497 grams of mercury in 700 ml. of nitric acid (sp. gr. 1.42) and diluting this solution with 2 volumes of water. One milliliter of the test solution is added to 2 ml. of Millon's reagent.

Phenol and o-cresol when present to about 1.0 mg. per ml., and m- and p-cresol when present to about 0.5 mg. per ml., produce a red color almost immediately at room temperature. When reduced to about 0.05 mg. per ml., each of these compounds produces in the cold or on careful heating a straw-yellow color which is destroyed by further heating.

MODIFICATION OF MELZER'S BENZALDEHYDE TEST (6) FOR DETECTION OF PHENOL AND o-, m-, AND p-CRESOL. Mix 1 ml. of the aqueous solution to be tested with 2 ml. of concentrated sulfuric acid (sp. gr. 1.84), and after cooling under the tap add 2 drops of benzaldehyde. Heat over a flame, cool, and add 10 ml. of water and 20 ml. of 40 per cent potassium hydroxide. The sensitivity of this test is about 1 mg. per ml. of solution.

MODIFICATION OF GUARESCHI'S TEST (4) FOR DISTINGUISHING PHENOL AND p-CRESOL FROM o- AND m-CRESOL. Add about 0.5 gram of solid potassium hydroxide and 3 drops of water to 3 ml. of a chloroform extract containing phenol or cresol, then warm and observe.

Straw-colored (yellow) globules will rise and the potassium hydroxide and water layer will assume a yellowish tinge on warming if phenol or p-cresol is present. In the presence of o- or m-cresol, the potassium hydroxide and the water layer will assume a pinkish or rose-red color. The sensitivity for each of these compounds is about 4 mg. in 3 ml. of extract.

FERRIC CHLORIDE TEST (8) FOR DETECTION OF o- AND p-

Table I. Color Changes

PHENOL	o-CRESOL	m-CRESOL	p-CRESOL
Changes observed in 5 minutes after addition of sulfuric acid and benzaldehyde			
Cloudy olive	Cloudy orange	Cloudy yellow	Milky white
After heating			
Cloudy reddish-brown	Cloudy brownish-red	Cloudy yellowish-brown	Cloudy brownish-green
After cooling and addition of water and potassium hydroxide			
Blue or violet solution and precipitate		Colorless or faintly tan colored solution and precipitate	



**CRESOL.** Add 2 drops of a freshly prepared 10 per cent aqueous solution of ferric chloride to 5 ml. of the test solution.

Phenol and *m*-cresol produce clear bluish-purple colors, *o*-cresol produces in about 10 minutes a slightly cloudy urine yellow or brown solution, while *p*-cresol produces a cloudy blue solution. The sensitivity for each compound is about 10 mg. in 5 ml.

**MODIFICATION OF LIEBERMANN'S TEST (5) FOR DETECTION OF *p*-CRESOL.** To 3 ml. of the unknown aqueous solution add slowly and with shaking 1 ml. of the reagent (6 per cent solution of sodium nitrite in concentrated sulfuric acid). A cloudy orange solution develops in about 5 minutes if *p*-cresol is present. Phenol and *m*-cresol yield clear, and *o*-cresol very slightly cloudy brown or yellowish-brown solutions. The sensitivity of this test is about 5 mg. in 3 ml.

**MODIFICATION OF COTTON'S TEST (1) FOR DETECTION OF *p*-CRESOL.** To 3 ml. of an aqueous solution, add 1 ml. of concentrated ammonium hydroxide (sp. gr. 0.901) and 4 drops of the freshly prepared reagent (10 ml. of concentrated hydrochloric acid and 0.5 gram of potassium chlorate added to 40 ml. of water).

Phenol and *o*- and *m*-cresol produce in 5 to 10 minutes clear light blue colors; *p*-cresol produces a clear light straw-yellow color. The sensitivity is about 10 mg. in 3 ml. of solution.

**HYPOCHLORITE TEST FOR DETECTION OF *o*-CRESOL.** To 5 ml. of the test solution, add one drop of sodium hypochlorite solution. In the presence of *o*-cresol the solution will immediately turn a cloudy yellowish-white; in the presence of phenol, and *m*- and *p*-cresol, it will remain clear and colorless. The sensitivity is about 4 mg. in 5 ml. of solution. (Excess of hypochlorite must be avoided because it may produce faint cloudiness with *p*-cresol.)

## DISCUSSION

It is reasonable to assume that all reagents discussed in this paper will also react with some compounds other than phenol, or *o*-, *m*-, or *p*-cresol. Therefore one must make certain that the test solution is comparatively free from compounds related to phenol or cresol. This may require preliminary precipitation, extraction, or distillation procedures.

Millon's test, even though it makes specific differentiation between phenol and the three cresols difficult, is of value because of its simplicity, as a preliminary test. This should be followed by the modified tests of Melzer and Guareschi, which will identify the compound. The conclusions drawn from these latter two tests may be checked by the hypochlorite and ferric chloride tests or by the modified procedures of Liebermann and of Cotton.

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# Stability of Standard Solutions of Copper Perchlorate and Potassium Iodate

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**W**HENEVER sodium thiosulfate is used in titration work of high accuracy frequent restandardization is necessary. In order to avoid the troublesome and wasteful necessity of preparing for each standardization a fresh solution of a primary standard [iodine, potassium iodate (3), copper perchlorate (2)], a standard in the form of a solution of a primary substance of dependable stability is desirable. The work reported here deals with the possibility of using potassium iodate or copper perchlorate (2) for such a purpose.

In considering the stability of such solutions it is necessary to distinguish between changes due to chemical instability, presumably resulting in decrease of active concentration, and changes due to evaporation from the container, which will cause increases of concentration. In the study of Berman (1), for instance, on the stability of potassium iodate, it is impossible to distinguish the role of these two factors. However, his data suggest that evaporation has been an appreciable factor in his results, and that, in some cases, an apparent stability has resulted from the opposing effects of evaporation and decomposition.

In a recent paper (4) on the stability of sodium thiosulfate solutions no account was taken of the possible effect of evaporation. A graphical study of the data on stability shows that in the first 60 days there is approximately a 0.3 per cent increase in normality. After that the values drop. Again two antagonistic tendencies, evaporation and decomposition, tend to produce a false picture of the stability of thiosulfate solutions.

The evaporation factor can be eliminated if, at the beginning of the experiment, samples of the solution to be examined are pipetted into separate flasks, and some of these are titrated at once, while others are titrated after a suitable lapse of time. The extent of evaporation, on the other hand, can be measured by

suitable weighings of the vessels containing stock solutions. If the solution is chemically stable, it is then possible to calculate the theoretical normality at any time from the initial normality and the loss of weight that has occurred.

## APPARATUS AND REAGENTS

A 50-cc. buret and one 10-cc. pipet were carefully calibrated and were used throughout the experiments. Details of the preparation and use of the copper perchlorate and potassium iodate are given in (2) and (3), respectively.

## EXPERIMENTAL

All titrations were carried out in duplicate. The amount of active substance present in solutions when they were fresh, and after they had stood for various lengths of time, was always determined by titration with thiosulfate (0.025*N*), newly standardized against two freshly prepared cupric perchlorate solutions (2)

Table I. Stability of 0.1*N* Copper Perchlorate and Potassium Iodate Solutions

Solution	Days Standing	Normality		Conditions
		Initial	Final <sup>a</sup>	
Cu(ClO <sub>4</sub> ) <sub>2</sub>	565	0.1007	0.1002	Thymol, glass stoppers
	565	0.1007	0.1004	Glass stoppers
	289	0.0993	0.0992	Cork stoppers, spores on cork
	454	0.0993	0.0993	
KIO <sub>3</sub>	565	0.1027	0.1021	Glass stoppers
	565	0.1027	0.1013	Thymol <sup>b</sup> , glass stoppers
	565	0.1027	0.0983	
	289	0.1007	0.1002	Cork stoppers, spores on cork
	454	0.1007	0.1004	

<sup>a</sup> Final normality calculated on basis of its initial volume.

<sup>b</sup> Only one sample; others are average value found for two samples.



Table II. Stability of Solutions in Glass-Stoppered Bottles

Days	Loss of Weight per 100 Days		Normality		Container	Bottle Capacity	Approximate Volume of Solution
	% of weight of solution present	Grams	Calculated	Found			
Copper Perchlorate							
0				0.1005	Glass-stoppered Pyrex about 12 years old	250	155
0-85	0.740	1.218	0.1011	0.1013			
85-290	0.679	0.898	0.1026	0.1025			
290-355	0.445	0.534	0.1033	0.1034			
0				0.1003	Glass-stoppered, flint	500	165
0-85	0.675	1.112	0.1009	0.1009			
85-290	0.488	0.671	0.1019	0.1023			
290-455	0.506	0.576	0.1028	0.1034			
0				0.1007	Rubber stopper, Pyrex	250	120
0-185	0.018	0.021	0.1007	0.1007			
0				0.0993	Glass stopper, Pyrex, sealed with paraffin	250	145
0-373	0.033	0.048	0.0994	0.0994			
3-1159	0.083	0.075	0.1001	0.0997			
Potassium Iodate							
0				0.1007	Glass stopper, flint glass; mold after several months	500	265
0-85	0.053	0.141	0.1007	0.1007			
85-453	0.095	0.156	0.1011	0.1010	Brown, glass stopper, paraffined	500	195
0				0.1028			
0-368	0.032	0.064	0.1029	0.1026			
368-768	0.021	0.036	0.1030	0.1021	Standard interchangeable glass stopper, Pyrex	250	160
0				0.1001			
0-157	0.051	0.083	0.1002	0.1000			
157-557	0.035	0.050	0.1003	0.0997	Glass stopper and ground-joint cap ("ether bottle")	500	185
0				0.1027			
0-238	0.021	0.040	0.1028	0.1027	Rubber stopper	250	165
0				0.1027			
0-165	0.022	0.036	0.1028	0.1013	Flint, glass stopper, turbid, deposit of inorganic material on walls	1 liter	85
0				0.1028			
0-855	0.130	0.113	0.1040	0.1037			

In the experiments summarized in Table I, 10-cc. samples of freshly prepared cupric perchlorate and potassium iodate solutions were pipetted into 125-cc. Erlenmeyer flasks. Some solutions were titrated at once, while other flasks were closed with either glass stoppers or fresh cork stoppers and protected against light by paper caps. These flasks were stored in the laboratory in a cabinet that was opened frequently and thus provided no protection against possible contamination from the laboratory atmosphere. The temperature was 22° to 35° C. The last of these solutions were titrated after 19 months. As a possible prevention of mold growth, about 10 mg. of thymol were added to some of the flasks.

Table III. Effect of Storage under Conditions of Minimum Evaporation

Solution	Days	Loss of Weight per 100 Days		Normality		Conditions
		% of weight of solution present	Grams	Initial	Final	
$\text{Cu}(\text{ClO}_4)_2$	368	0.018	0.019	0.1022	0.1018	Glass stopper, Pyrex
$\text{KIO}_3$	392	0.0018	0.0018	0.1002	0.0999	Glass stopper, flint

Table I shows the results. It appears that copper perchlorate solutions possess a high degree of stability, while potassium iodate solutions show a pronounced tendency to become weaker. In the latter case thymol has a harmful effect, presumably because it is acidified, while in the copper perchlorate solutions, even the presence of black, sporelike spots on the cork stoppers was not associated with any loss of titer.

In the experiments summarized in Table II, samples of freshly prepared copper perchlorate and potassium iodate solutions were treated with thiosulfate. The remaining portions of the solutions were transferred to clean, dry, tared, glass-stoppered reagent bottles and weighed with an accuracy of  $\pm 5$  mg. After standing for varying periods under the conditions described above, the bottles were carefully dusted and about half an hour later weighed. A pair of 10-cc. samples were withdrawn and

titrated as above. The bottles were then reweighed and the above procedure was repeated at intervals. From the data given, one can readily calculate the initial weight of the solutions. In some cases, portions of the solutions were removed for other purposes. The bottles were weighed before and after such removals and due corrections were applied in the calculations.

In order to keep evaporation at a minimum the following procedure was tried. A tared 250-cc. glass-stoppered bottle containing about 100 cc. of copper perchlorate solution was weighed. The bottle, placed in a dry beaker, was stored in a desiccator over a portion of the same solution. One year later the bottle was taken from the desiccator, wiped, and carefully weighed as before. Duplicate 10-cc. samples were then titrated. A potassium iodate solution was treated in the same manner. A comparison of the results obtained (Table III) with those shown in Table II shows that by the use of good glass-stoppered or rubber-stoppered bottles nearly the same results can be attained as by the desiccator method.

### CONCLUSIONS

Solutions in glass-stoppered bottles may lose weight by evaporation. This loss in weight has a tendency to give some types of solutions an appearance of stability. Results of experiments indicate that solutions of copper perchlorate are more stable than those of potassium iodate. For use as permanent standards copper perchlorate solutions should be stored in good glass-stoppered or rubber-stoppered bottles (evaporation losses should be determined by weighing, and normalities should be corrected accordingly). Iodate solutions must be stored in glass-stoppered bottles. Thymol should not be used as a preservative for either copper perchlorate or potassium iodate solutions.

### ACKNOWLEDGMENT

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# Semiautomatic Pressure Control in Low-Pressure, Low-Temperature Laboratory Fractionation

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THE necessity of using laboratory personnel manpower to the best advantage and the desire continually to improve the quality of fractional analysis have led to the installation of automatic (2) and semiautomatic control (1, 2, 4) on fractionating columns in many laboratories. In the past, even semiautomatic pressure control (2, 3) has entailed the use of an elaborate device installed at no small expense. The pressure control devices described by Bosschart (1) and Podbielniak (2) employ compressed air for dispensing liquid nitrogen to the column head by means of electrically operated valves controlled by electrical contacts in the column manometer.

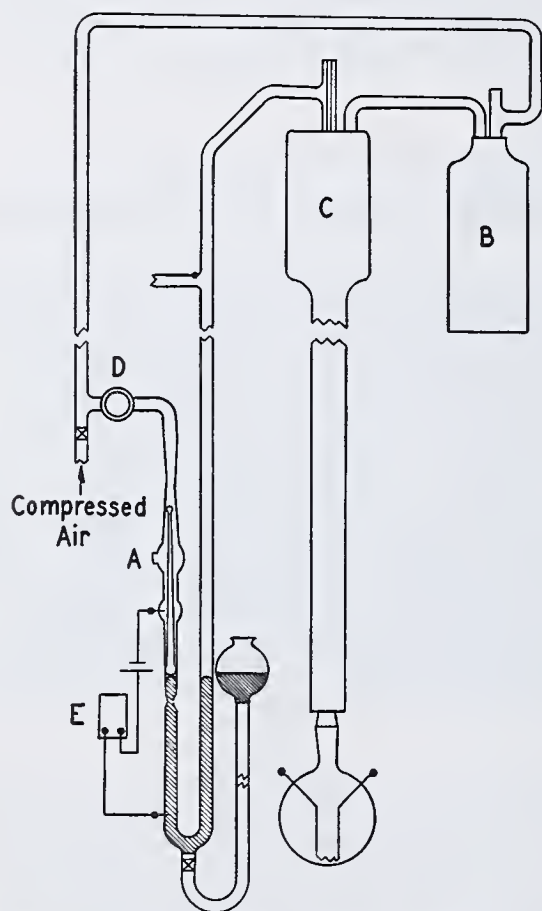


Figure 1. Semiautomatic Pressure Control Device

- A. Throttling valve
- B. Liquid nitrogen dispenser
- C. Column condenser
- D. Manual control
- E. Electrical buzzer

This paper describes a control device developed in this laboratory from materials usually available, which provides a simplified means of semiautomatic control for low-temperature, low-pressure laboratory fractionating equipment, without sacrificing excellence of control or ease of operation. Its outstanding attributes are simplicity of construction and low cost. The device may be used with good results on either the standard low-temperature laboratory fractionating column or the Podbielniak Heli-Grid type (3); and if these two types of columns are connected to the same manifold, the control may be shifted from one column to the

other simply by moving the nitrogen flask. No other change in the pressure control mechanism are necessary.

The principal features of the control device are shown in Figure 1, in relation to the fractionating column. Three essential parts of the mechanism are shown in detail: the throttling valve in Figure 2, and the nitrogen dispenser and nitrogen pander in Figure 3.

## THROTTLING VALVE

The throttling valve (Figure 2) is an extension of the outer arm of the column manometer, and is actuated by mercury which rises out of the manometer and into the valve, causing the needle, *a*, to float on the meniscus of the mercury column. The ball on top of the needle will move in the throat, *b*, of the valve with the rise and fall of mercury. This motion will restrict the flow of a stream of air which normally exhausts through the vent on the valve and must, as a result of the throttling effect of the needle, create sufficient pressure within the nitrogen-dispensing bottle to cause a discharge of liquid nitrogen. The needle seeks an equilibrium position in the throat of the valve, effecting a small but constant discharge of nitrogen just sufficient to control the pressure in the column and maintain it with very slight fluctuation. The amount of mercury in the manometer may be varied to produce any pressure plateau desired from 0 to 760 mm.

The dimensions of the throttling valve are somewhat arbitrary; but 7-mm. glass tubing for the column manometer and lower section of the control valve in which the needle rides was found to give satisfactory results. The following dimensions are suggested: length of throat section, 7.5 cm.; diameter at narrowest section of throat, 2 to 2.5 mm.; length of needle, 9 cm. The throat section can be drawn down to proper size from 10- or 11-mm. glass tubing; the main precautions are to keep the glass circular and as thick as possible. After a satisfactory throat section has been drawn, it should be sealed to tubing of the proper size (7 mm.). The ball on top of the needle should just pass through the narrowest section of the throat without sticking, and the bottom of the needle must ride freely in the tube on the mercury meniscus. The most satisfactory method for connecting the throttling valve to the column manometer is shown in Figure 4. The three-way stopcock should be at least 25 mm. below the 760-mm. mark on the meter stick to allow a length of manometer tube to take care of pressure build-up when recharging the nitrogen bottle.

The needle in the throttling valve described above never seats, but allows exhaust air to flow through at all times. The movement of the needle in the tapered section varies the size of the orifice formed by the throttling valve, which acts to control the flow of exhaust air, thereby producing control of pressure within the nitrogen-dispensing system. The position of the ball on the needle in the lower tapered section of the throat of the valve restricts the flow of air through the valve just enough to create sufficient pressure within the nitrogen-dispensing system to force the amount of liquid nitrogen out of the liquid nitrogen flask needed to control column pressures and to maintain liquid reflux in the column. As the nitrogen level in the flask decreases, more

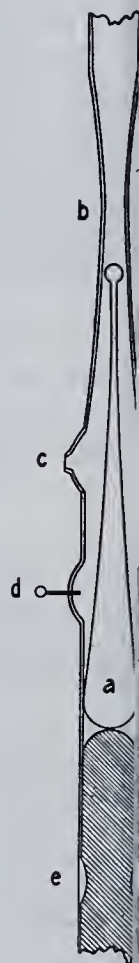


Figure 2. Throttling Valve

- a. Floating needle
- b. Throat section
- c. Air exhaust
- d. Tungsten
- e. Constriction tubing



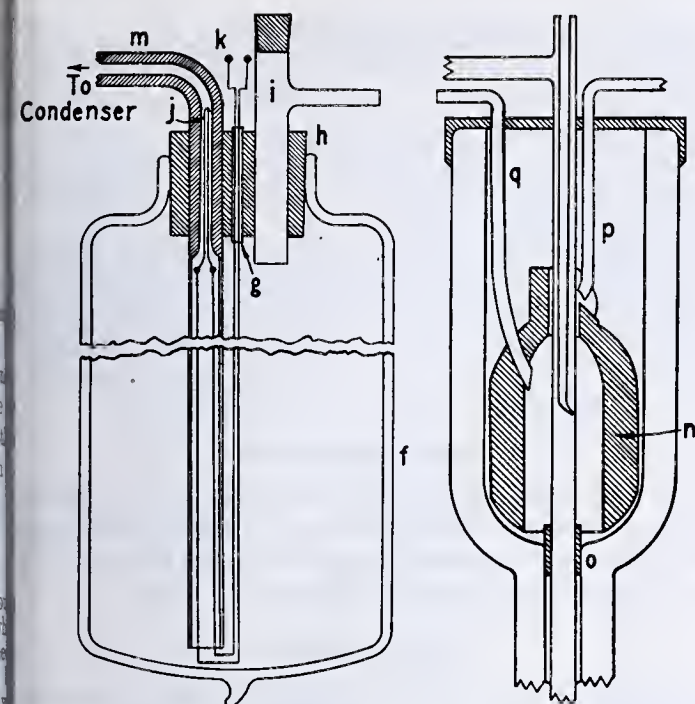


Figure 3. Liquid Nitrogen Dispenser (left) and Nitrogen Expander (right)

- |                          |                                |
|--------------------------|--------------------------------|
| f. Dewar flask           | m. Discharge tube              |
| g. Porcelain conduit     | n. Nitrogen expander           |
| h. Rubber stopper        | o. Asbestos string packing     |
| i. Air intake tube       | p. Liquid nitrogen intake tube |
| j. Heating element       | q. Spent nitrogen exhaust tube |
| k. Electrical lead wires |                                |

Pressure is required in the flask to force the liquid nitrogen up; this extra pressure is furnished automatically by the valve, since the ball on the needle merely assumes a position further up the throat, further restricting the flow of air. Within reasonable limits, the rate of furnishing compressed air to the nitrogen-dispensing system produces no difficulties to operation, since the needle in the throttling valve automatically adjusts itself to a position suitable for operation at a given rate of air flow. The rate of air flow is usually set to permit the ball of the needle to operate at a point about 1 to 1.5 cm. below the narrowest section of the throat. Since the nitrogen expander has been designed to permit rapid response of column pressure to the addition of cooling agent, the throttling valve is capable of automatically controlling the column pressure within very narrow limits of fluctuation.

#### LIQUID NITROGEN DISPENSER

The nitrogen-dispensing unit shown in detail in Figure 3 (left) contained in a quart-size, wide-mouthed Dewar flask. An air intake tube, *i*, a nitrogen discharge tube, *m*, and electrical leads, *k*, are sealed into the flask by means of a rubber stopper, *h*, and a porcelain conduit, *g*, for the electrical lead wires. Air which has been induced to flow into the Dewar flask as a result of the action of the throttling valve will force liquid nitrogen up into contact with the heating element, *j*, which being relatively small flash-vaporizes a small portion of the liquid nitrogen, and the sudden expansion of the vapor forcibly ejects the liquid nitrogen from the discharge tube. The increments of liquid nitrogen discharged in this manner are of small size, since the flash-vaporization occurs before any large quantity can find its way into the upper portion of the discharge tube. The heating element in the discharge tube will continue to expel liquid nitrogen in small bursts, at rapid, regular intervals, which are regulated as regards amount of nitrogen and time interval by the pressure brought to bear by the throttling valve, which in turn reflects the need of the column for cooling agent. The heating element can be made from 8.75 cm. (3.5 inches) of No. 26 Chromel resistance wire, sufficient to make a heater which extends from just below the rubber stopper to the bend at the top of the discharge tube. During an analysis it is necessary to vary the amount of current in the heating element; this can be done with a small 2-ohm, 5-ampere rheostat if a potential of 6 to 9 volts is being used. The discharge tube, *m*, should be made of 9-mm. glass tubing sealed as it enters the rubber stopper with capillary tubing having 2.5- to 3-mm. inside diameter. Capillary tubing smaller than 2.5 mm. cannot be used successfully,

as the large resistance offered to the flow of nitrogen makes it necessary to maintain an unduly large pressure in the Dewar flask in order to force over sufficient nitrogen.

The heating element in the liquid nitrogen dispenser is useful for reducing the fluctuation in column pressure during the fractionation of methane and ethane, since it tends to cause delivery of the cooling agent to the column head in the form of a more nearly continuous stream of dropwise increments of liquid nitrogen automatically adjusted to meet the column requirements. For separating the components propane through hexane the throttling valve usually affords satisfactory control of column pressure. The use of the heating element in the liquid nitrogen dispenser appears to cause very little if any significant increase in the liquid nitrogen requirements for fractionating methane and possibly ethane. Since only a small portion of the liquid nitrogen is vaporized by the heater while fractionating methane,

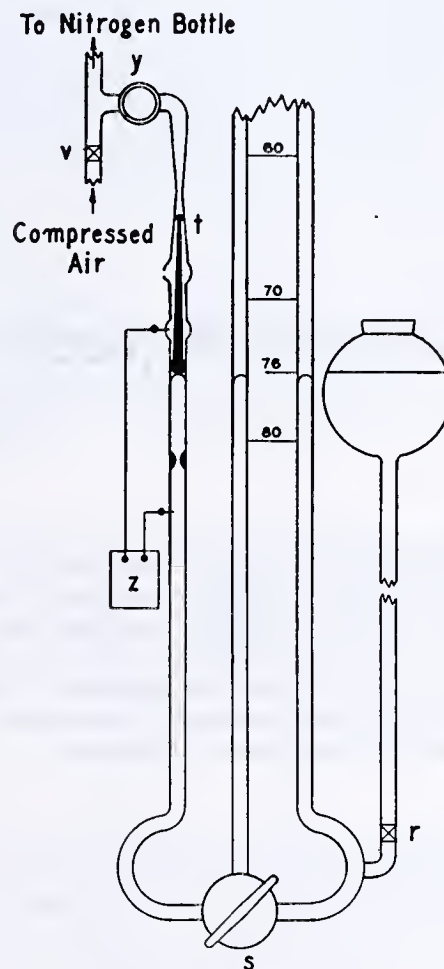


Figure 4. Connection of Throttling Valve to Column Manometer

- |                                 |
|---------------------------------|
| r. Pinch clamp                  |
| s. Three-way stopcock           |
| t. Throttling valve             |
| v. Valve on compressed air line |
| y. Manual control button        |
| z. Electrical buzzer            |

the more uniform manner of dispensing nitrogen to the column head enables better operation of the column and more efficient use of the cooling agent, thereby partially compensating for the nitrogen requirements of the heater.

#### NITROGEN EXPANDER

In designing the nitrogen expander (Figure 3, right) and the nitrogen intake tube, it is important to keep in mind that the liquid nitrogen should be conducted from the Dewar flask to make contact with the distilling tube by the shortest possible



route and should in no case pass through a difficult path before making contact. The intake tube, *p*, should be a continuation of tube *m* in the nitrogen flask, and should have the same inside diameter (2.5 mm.). Expander *n* is machined from a solid piece of metal, and is supported by small pieces of rubber or felt inside the column condenser. This type of expander allows the nitrogen to contact the distilling tube immediately, and conducts any excess nitrogen away from the distilling tube where it may vaporize without causing excessive cooling near the tube. The packing, *o*, is asbestos string wound around the distilling tube and pressed into place. Precautions must be taken to prevent water from accumulating in the nitrogen expansion chamber or around the distilling tube.

#### TECHNIQUE OF OPERATION

During the time that a sample is introduced into the fractionating column, the throttling valve controller should be cut out by turning the stopcock (*s*, Figure 4). After sampling is completed and the kettle allowed to warm up, clamp *r* should be released from the leveling bottle and may be closed again when the desired pressure is reached. At some time during the period when pressure is building up, valve *v* should be opened, causing a small stream of air to flow through the throttling valve. The condenser head on the column is next brought to proper temperature, using the manual control button, *y*, and should be at temperature when the throttling valve controller is cut in.

Soon after the throttling valve controller is cut in, the nitrogen bottle should be inspected for leaks around the stopper and special care taken to see that a smooth connection is made between the discharge tube in the nitrogen bottle and the tube to the column head.

If the sample being analyzed contains methane, it may be desirable to build up reflux in the column with the manual control button, and cut in the throttling valve controller after the column has become completely wet.

An operator must be present to adjust the take-off rate valve at the various cut points and to record the necessary data. The buzzer, *z*, signals the operator when the nitrogen bottle is empty, or if for any other reason the column condenser is not being supplied with sufficient nitrogen. With a suitable arrangement of laboratory equipment it is frequently possible for one operator to operate two columns simultaneously.

#### ACKNOWLEDGMENTS

The authors are indebted to T. A. Matthews, C. F. Weinert, and D. E. Smith of Phillips Petroleum Company Laboratory for assistance or suggestions and to the Phillips Petroleum Company for permission to publish this paper.

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## Color of Aqueous Potassium Dichromate Solutions

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**A spectrophotometric study has been made of the color of aqueous potassium dichromate solutions, including the effect of pH and of the concentration of acid, base, and dichromate. All these variables have an effect on the color of the solutions, and any recommendations of potassium dichromate solutions as permanent colorimetric standards should specify the exact nature of the solution.**

**A**QUEOUS solutions of potassium dichromate and/or potassium chromate are recommended as permanent colorimetric standards in a number of colorimetric methods involving unstable yellow colors. Examples are the following procedures: silica as molybdisilicic acid, residual chlorine in water, carotene, and varnish. In spite of these widespread uses, little work has been done on the color of potassium dichromate and potassium chromate solutions, especially the effect on them of such variables as pH and the kind and amount of acid or base added.

Hantzsch and Clark (3), Neuss and Rieman (4), Sherrill (5), Vosburgh and Cooper (7), and others have studied the chromate-dichromate relationship from the standpoint of the ionic equilibria involved rather than the color of the solutions. Swank and Mellon (6) pointed out the necessity of buffering potassium chromate solutions at pH 9 to secure color matches with molybdisilicic acid.

The present spectrophotometric study was undertaken to determine the effect of pH, various acids and bases, and dichromate concentration on the color of aqueous potassium dichromate solutions.

#### EXPERIMENTAL WORK

**APPARATUS AND SOLUTIONS.** Transmittancy measurements were made in 1.000-cm. cells with a General Electric recording

spectrophotometer, adjusted for a spectral band width of either 5 or 10  $\mu$ . In case of colored reagents, these solutions were used in the reference cell. Otherwise, redistilled water served. All pH measurements were made with a glass electrode assembly.

Stock solutions of potassium dichromate were prepared from twice recrystallized salt and redistilled water. Two solutions containing 10.00 grams per liter and 12.50 grams per 100 ml. were used. A series of Clark and Lubs buffer solutions was prepared according to the directions of Clark (2).

**EFFECT OF pH ON THE COLOR OF POTASSIUM DICHROMATE SOLUTIONS.** To study the effect of pH on the chromate-dichromate system, the desired amount of potassium dichromate solution was pipetted into a 50-ml. volumetric flask, and then diluted to the mark with a buffer of the desired pH. The contents were mixed and allowed to sit a few minutes to ensure equilibrium. The spectral transmission curve and the pH were then determined.

In general, dichromate solutions at a low pH have an orange hue, while those at a high pH are yellow. These colors are commonly associated with the dichromate and chromate ions, respectively. An intermediate range exists in which the hue is extremely sensitive to small changes in pH. At low dichromate concentrations this intermediate range extends from pH 5 to 8, as shown in Figure 1. At higher concentrations, the range shifts upward, being from pH 6 to 8 at 2.0 mg. of potassium dichromate per ml.

At low concentrations of salt the series of solutions at various pH values has an isobestic point (1) (Figure 1). With different concentrations of dichromate, the transmittancies of this point vary according to Beer's law. The wave length at this point increased from 444  $m\mu$  at 0.2 mg. to 440  $m\mu$  at 1.0 mg. of dichromate per ml. At concentrations greater than 1.0 mg. per ml., absorption is too high at these wave lengths to show the point.

**EFFECT OF ACIDS.** In studying the effect of various acids, in all subsequent work, the following procedure was used:



The dichromate solution was pipetted into a 50-ml. volumetric flask, the acid or base added, and the solution diluted to the mark at room temperature with water. In case the water could not be added to the concentrated acid, approximately the desired amount was added to the flask before the acid. The transmittancy curves were determined within an hour after making the final volume adjustments.

With single acids two dichromate concentrations were used: 4 and 5.0 mg. per ml. The former was low enough to show the relatively flat portion of the transmittancy curve near 440  $m\mu$ , and to observe the effect of acid upon it. The latter was high enough so that this part of the curve did not appear. The acid concentration varied from none to concentrated acid.

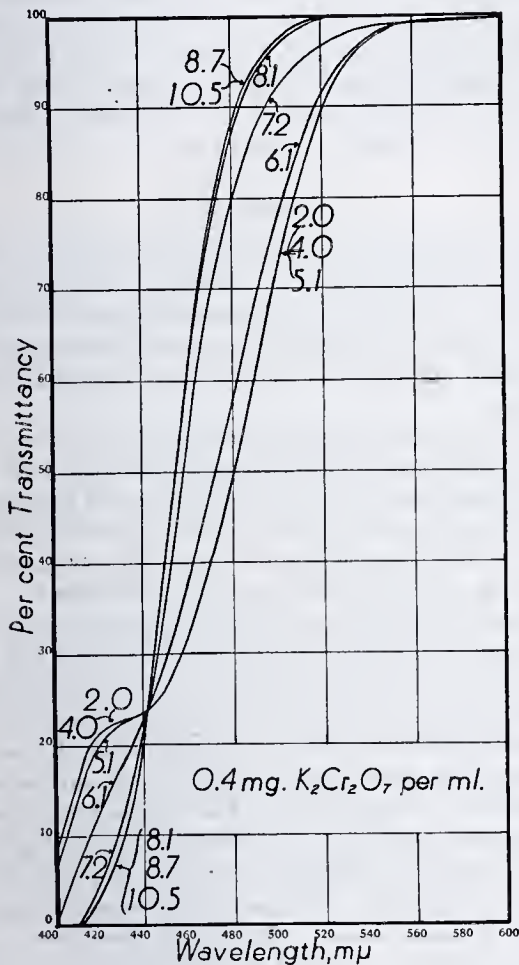


Figure 1. Effect of pH on the Color of Potassium Dichromate Solution

pH on curve

With mixed acids only one concentration of dichromate was used: 0.4 mg. per ml. The total acid concentration did not exceed that likely to be encountered in general analytical practice. With the binary mixtures 5 ml. of each acid per 50-ml. final volume was the maximum, while with the ternary and quaternary mixtures the maximum of each acid was limited to 3 ml.

Lack of space prevents presentation or adequate description of all the transmittancy curves. An attempt is made in the following paragraphs to summarize their characteristics:

**Sulfuric Acid.** Potassium dichromate solutions containing sulfuric acid show the greatest variation in color of any of the acids used. With a low concentration of dichromate, small amounts of acid give a very definite minimum in the curve near 440  $m\mu$ , the wave length of the minimum being related to the acidity (Figure 2). Larger amounts of acid, up to 9M, cause no great change. Between 9 and 11M, however, the hue of the solutions changes from orange to orange-red, and the minimum point in the curve disappears. As the acidity is increased further, there is a shift toward an orange-brown hue, with no striking change in the curve.

A high concentration of dichromate behaves similarly, but there is no minimum in the curve at concentrations much greater than 1 mg. of dichromate per ml. because of complete absorption

where the minimum should be. Even a small amount of acid shifts the entire curve toward longer wave lengths. Then acidities on up to 9M have little effect on the color; but between 9 and 12M the color changes from orange-red to brown. Above 12M there is little further color change.

**Nitric Acid.** With a low dichromate concentration solutions containing nitric acid show the first of the two color changes noted with sulfuric acid-potassium dichromate solutions. The minimum near 440  $m\mu$  appears when the acidity reaches 5M, and the intensity of the minimum increases with increasing acidity. With a high dichromate concentration the entire curve shifts to longer wave lengths as the nitric acid concentration increases. The brown color noted with sulfuric acid does not appear.

**Phosphoric Acid.** Qualitatively phosphoric acid has much the same effect on the dichromate color as nitric acid, but quantitatively it differs considerably. With a low dichromate concentration the minimum near 440  $m\mu$  is definite with 0.3M acid. Increasing the acidity to 1.0M increases the intensity greatly, but higher acidities cause little further change. The transmittancy curve for high dichromate concentrations shifts to longer wave lengths with small amounts of acid, but, as before, amounts greater than 1.0M have little additional effect.

**Perchloric Acid.** With a low dichromate concentration, this acid has little effect on the color. The minimum in the transmittancy curves does not appear, but the percentage transmittancy of the horizontal portion of the transmittancy curve increases slightly with increased acid concentration. In the presence of larger amounts of potassium dichromate, a precipitate, presumably potassium perchlorate, appears.

**Acetic Acid.** Dichromate solutions containing acetic acid show changes similar to those with nitric acid. The magnitude of the changes is greater than those with nitric acid, although not so large as the corresponding changes with sulfuric acid.

**Hydrochloric Acid.** Although it is generally assumed that potassium dichromate does not oxidize hydrochloric acid in aqueous solution, the yellow color of solutions which contain 0.4 mg. of potassium dichromate per ml. and which are 9M or stronger in acid disappears within 5 minutes after addition of the acid.

Because of this reaction, which proceeds at a slower rate in more weakly acidic solutions, hydrochloric acid solutions of potassium dichromate fade and should not be used as permanent

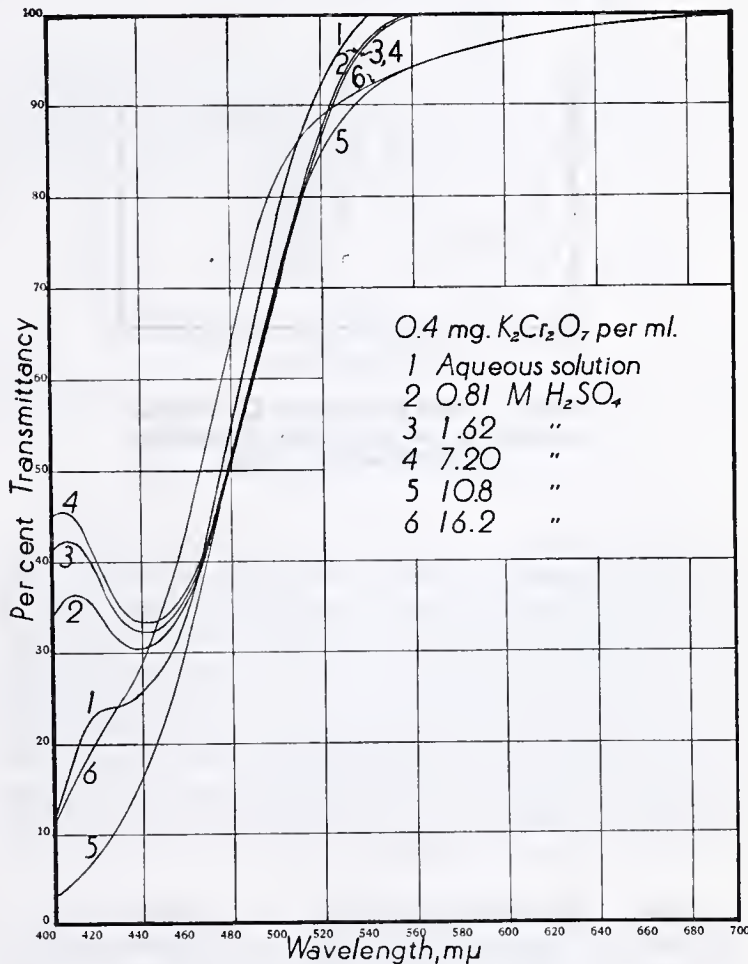


Figure 2. Effect of Sulfuric Acid on the Color of Potassium Dichromate Solutions



standards. A solution 0.8M in acid fades about 2 per cent in 48 hours, and one 0.4M in acid fades to the same extent in a month.

If the fading of the solution is overlooked, hydrochloric acid affects the color in a manner similar to nitric acid. The minimum appears at 0.4M acid, and as the acid concentration increases the minimum is more marked.

**Binary Mixtures.** Binary mixtures containing small amounts of phosphoric acid and one of the other acids show very definite minima near 440 m $\mu$ . Changes in the concentration of the second acid have only a small effect on the color of the solution. The minimum is present in the transmittancy curve even if the second acid is nitric or perchloric acid, neither of which, alone and at low concentrations, causes the minimum to appear.

Mixtures of either perchloric or nitric acid and sulfuric acid show limited minima formation at low, and definite minima at higher, sulfuric acid concentrations.

Mixtures of nitric and perchloric acid show no minima in the transmittancy curve.

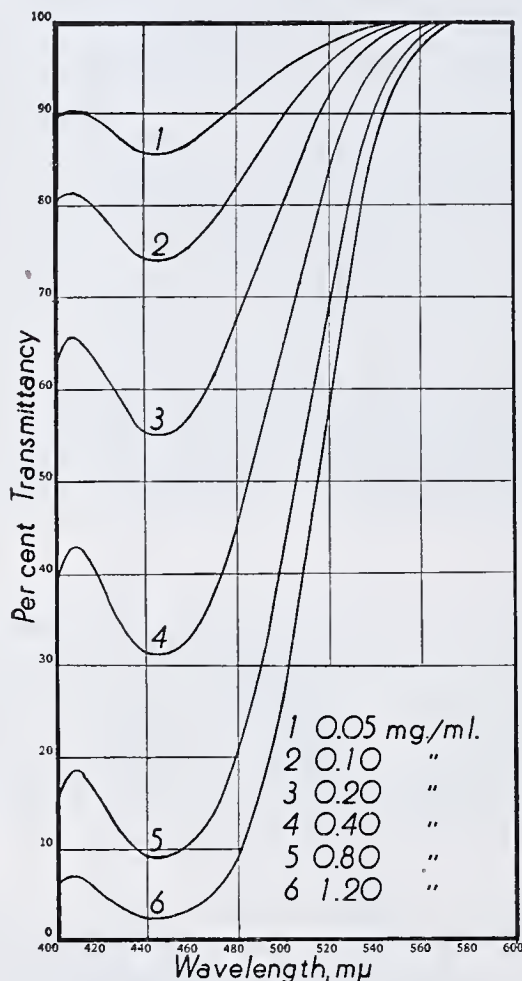


Figure 3. Effect of Potassium Dichromate Concentration on the Color of Solutions in 1.5M Phosphoric Acid

**Ternary Mixtures.** Solutions containing small amounts of phosphoric acid with two other acids show a very definite minimum near 440 m $\mu$  in the transmittancy curve. The intensity of the minimum is not much affected by small changes in the concentration of either or both of the other acids.

Solutions not containing phosphoric acid show a limited minimum formation, and the intensity of the minimum is affected by changes in the concentration of the other acids. Increases in sulfuric acid concentration increase the minima, while increases in nitric and/or perchloric acid decrease them.

**Quaternary Mixtures.** The spectrophotometric curves for all the quaternary mixtures tested were virtually the same, and all showed a pronounced minimum near 440 m $\mu$ .

**EFFECT OF DICHROMATE CONCENTRATION.** The range of concentration for measurements of dichromate solutions in 1-cm. cells depends on the amount and kind of acid used, and the wave length at which the measurements are made. With 1-cm. cells, measured at the wave length of minimum transmission, 0.02 to

1.2 mg. of potassium dichromate per ml. can be determined in solutions 1.8M in sulfuric acid or 1.5M in phosphoric acid (Figure 3). Beer's law is valid over the entire range in both cases. With 1.6M nitric acid, 1.7M acetic acid, or 0.9M perchloric acid, 0.02 to 0.9 mg. of potassium dichromate per ml. can be determined if the measurements are made in 1-cm. cells at 430 m $\mu$ . Beer's law is valid only to 0.6 mg. per ml. for these solutions.

If the dichromate solution is adjusted to pH 2 or 9, and the transmittancy measurements are made in 1-cm. cells at 440 m $\mu$ , the measurable range is 0.02 to 1.0 mg. per ml., Beer's law being valid over the entire range.

**EFFECT OF BASE.** In this study the same experimental procedure was followed as with the acids. A freshly prepared solution, 8M in sodium hydroxide, served as the source of the base.

After enough base has been added to convert the dichromate to chromate, addition of an excess has little effect. Solutions 8M in base show a very slight greenish tint.

## DISCUSSION

Solutions of potassium chromate and/or potassium dichromate used as permanent colorimetric standards should be buffered to a definite pH. In the case of the intermediate pH values, the buffer should have a high capacity, since small changes in pH make a large change in the color. At either a high or low pH this is not so important.

It is necessary to specify both the kind of acid, or acids, and the amount of each when acidified dichromate solutions are used. If the phosphoric acid is more than 0.3M, small variations in the concentration of a second, third, or fourth acid will not seriously change the color of the solution. Solutions containing hydrochloric acid are not suitable for permanent standards.

A high concentration of base should be avoided, since it attacks glass and causes turbidity. A low concentration (pH 9 or 10) gives the same color, with much less attack on the container.

The isobestic points probably have some significance in terms of the ionic equilibria involved. Clark (1) states that an isobestic point is an intersection of all isohydric curves, and "consequently the probability of occurrence is low unless two colored compounds and two only have some intimate relationship". Such a point in the chromate-dichromate transmission curves seems to indicate that only two ions are significantly involved in the equilibrium transformation. Three ions have been postulated for this system,  $\text{CrO}_4^{--}$ ,  $\text{HCrO}_4^-$ , and  $\text{Cr}_2\text{O}_7^{--}$ , the reactions involved being



The isobestic point would seem to indicate that Reaction 1 is of major importance, since it alone involves hydrogen ions. Calculations from the data of Neuss and Rieman (4) show that 88 per cent of the dichromate ion in a solution containing 1 mg. of potassium dichromate per ml. should react to give the  $\text{HCrO}_4^-$  ion. If these data, and the facts about isobestic points, are correct, the absorption spectra of the  $\text{HCrO}_4^-$  and  $\text{Cr}_2\text{O}_7^{--}$  ions should be similar, since conversion of one form to the other has little effect on the over-all transmittancy.

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ABSTRACTED from a thesis presented by R. E. Kitson to the Graduate School of Purdue University in partial fulfillment of the requirements for the degree of doctor of philosophy, February, 1944.



# Gravimetric Determination of Tungsten

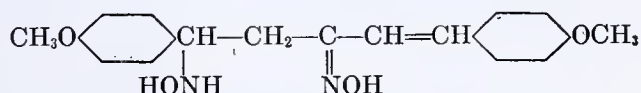
**With Anti-1,5-di-(*p*-methoxyphenyl)-1-hydroxylamino-3-oximino-4-pentene**

JOHN H. YOE AND A. LETCHER JONES<sup>1</sup>

University of Virginia, Charlottesville, Va.

A new organic compound has been developed as a reagent for the gravimetric determination of tungsten. Its physical and chemical properties have been investigated and procedures are given for its use in the determination of tungsten in ores and alloys. Determinations of tungsten with the new reagent are equivalent in accuracy to the standard cinchonine method.

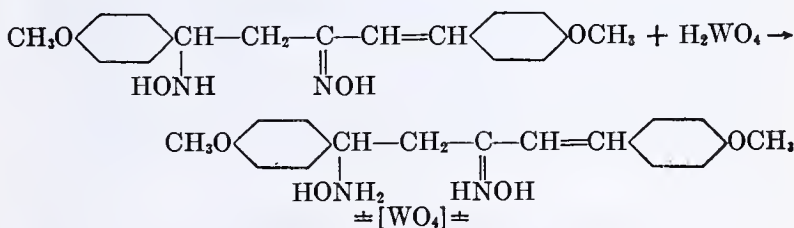
pressly for this work, was observed to form a highly insoluble yellow precipitate with tungstate ions ( $\text{WO}_4^{--}$ ) in acid solution. A detailed investigation revealed that this new compound combines with the tungstate ion



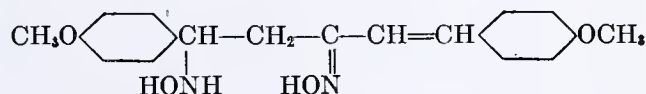
in a definite ratio of one molecule of reagent to one tungstate ion and that this reaction may be applied to the gravimetric determination of tungsten.

### NATURE OF REACTION WITH TUNGSTATE ION

The exact mechanism by which the reagent combines with the tungstate ion to form the insoluble complex is not known with certainty. It is possibly a salt-forming reaction:



The reagent belongs to the basic salinogenic group of organic compounds, many members of which combine with anions such as nitrates, perchlorates, sulfate, phosphates, molybdates, vanadates, thiocyanates, etc. (28), in acid medium. The "syn" isomer of the organic compound is completely unreactive with the



tungstate ion. It crystallizes in the form of colorless plates (m.p. 217° C.).

### PHYSICAL AND CHEMICAL PROPERTIES OF REAGENT

The reagent crystallizes from ethanol in the form of small yellow needles which melt at 156–157° C. (corrected). It is soluble in the following organic solvents, solutions being yellow in all instances: ethanol, acetone, ethyl ether, ethyl acetate, dioxane, acetic acid, and benzene. The compound is insoluble in water and petroleum ether. Solutions of the reagent in ethanol are used in tungsten analyses. The solubility is 0.766 gram of reagent per 100 ml. of ethanol at 25° C.

**SOLUBILITY OF TUNGSTEN COMPLEX.** An experiment was conducted to determine whether or not the precipitated organotungsten complex was soluble in water to the extent of one part per million.

One milligram of finely pulverized dried complex (dried at 105° C. for 2 hours) was introduced into a flask containing 1 liter of water at 25° C. The water was mechanically stirred for one week. At the end of that time, the complex had not dissolved; hence it was concluded that its solubility is less than 1 mg. per liter of water—i.e., 1 p.p.m.

**M**OST procedures for the gravimetric determination of tungsten involve separation of the major portion of the element from solution as tungstic acid,  $\text{H}_2\text{WO}_4$ , by strong mineral acid treatment, and recovery of the small amount remaining in solution by means of an organic precipitant. Cinchonine is generally used for this purpose. Present governmental restrictions on the sale of cinchonine for analytical purposes and the increasing number of applications of tungsten and tungsten alloys to industrial uses make the introduction of an effective and easily obtainable reagent for tungsten particularly desirable at this time.

This paper describes a new organic reagent for tungsten, *anti*-1,5-di-(*p*-methoxyphenyl)-1-hydroxylamino-3-oxo-4-pentene, first synthesized in this laboratory and applied to the analysis of a variety of tungsten ores and alloys with highly satisfactory results. The compound may now be obtained from **AMOTTE Chemical Products Co., Towson 4, Baltimore, Md.**

Various organic reagents for the gravimetric determination of tungsten have been proposed since Lefort (13) first reported in 1881 that quinine acetate would precipitate tungstates from solution. Cremer (1) reported the cinchonine reaction in 1895, but it was not applied to actual analyses until about 20 years later when Low (14) developed a gravimetric procedure employing cinchonine as a precipitant for tungsten. Jannasch and Bettendorfs (8) used hydrazine hydrochloride and strong hydrochloric acid to precipitate tungsten but separations were not complete by this method. Knorre (11) found benzidine hydrochloride slightly better than hydrazine hydrochloride but still not entirely satisfactory.

Other investigators have proposed a variety of gravimetric organic reagents:  $\alpha$ -naphthylamine and cumidine (23), tetraethyl-*p*-diaminodiphenylmethane (10, 20), 1,4-diphenylendanioldihydrotriazole (Nitron) (4), quinoline (15), tannin (16, 21), phenylhydrazine hydrochloride (2), 8-hydroxyquinoline (5, 9, 18), anillylidene-benzidine (7), and rhodamine B (17). None of these reagents has proved sufficiently effective to replace cinchonine as the preferred reagent in standard procedure, although its use involves certain difficulties (1, 6, 12).

Early in 1931 a systematic investigation of the reactivity of organic compounds, with respect to color and precipitate formation with inorganic ions, was begun in this laboratory. Standard solutions of about eighty inorganic ions were prepared and tested, in both acid and alkaline medium, with various types of organic compounds, especially those with one or more chelating or salt-forming groups. The oximes, as a class, appeared to be one of the most promising types to try in this search for specific and highly sensitive reagents for inorganic ions.

During the 1930's several new and important organic analytical reagents were discovered in this laboratory (19, 24-27). In 1940 anti-1,5-di-(*p*-methoxyphenyl)-1-hydroxylamino-3-oxo-4-pentene, one of a series of compounds synthesized ex-

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**STABILITY OF REAGENT TO LIGHT.** The pure reagent is pale yellow in color but is slowly darkened by light. This discoloration does not affect its reactivity with tungstates. No discoloration occurs if stored in dark bottles.

**REACTIONS WITH INORGANIC IONS.** Tests for reactivity with inorganic ions were made on porcelain spot plates by adding a drop of an ethanol solution of the reagent to a drop of solution containing the respective ions (approximately 0.05 mg.) in both acid and alkaline medium where possible. No reactions were observed between the reagent and any of the following ions:

$\text{Ag}^+$ ,  $\text{Al}^{+++}$ ,  $\text{As}^{+++}$ ,  $\text{AsO}_4^{---}$ ,  $\text{B}_4\text{O}_7^{--}$ ,  $\text{Ba}^{++}$ ,  $\text{Be}^{++}$ ,  $\text{Bi}^{+++}$ ,  $\text{Br}^-$ ,  $\text{CO}_3^{--}$ ,  $\text{Ca}^{++}$ ,  $\text{C}_2\text{O}_4^{--}$ ,  $\text{Cd}^{++}$ ,  $\text{Ce}^{+++}$ ,  $\text{Cl}^-$ ,  $\text{Co}^{++}$ ,  $\text{Cr}^{+++}$ ,  $\text{Cs}^+$ ,  $\text{Dy}^{+++}$ ,  $\text{Er}^{+++}$ ,  $\text{Eu}^{+++}$ ,  $\text{F}^-$ ,  $\text{Fe}^{++}$ ,  $\text{Ga}^{+++}$ ,  $\text{Gd}^{+++}$ ,  $\text{Ge}^{++++}$  (aqueous solution of  $\text{GeO}_2$ ),  $\text{HfO}^{++}$ ,  $\text{Hg}^+$ ,  $\text{Hg}^{++}$ ,  $\text{I}^-$ ,  $\text{In}^{+++}$ ,  $\text{K}^+$ ,  $\text{La}^{+++}$ ,  $\text{Li}^+$ ,  $\text{Mg}^{++}$ ,  $\text{Mn}^{++}$ ,  $\text{NO}_2^-$ ,  $\text{NO}_3^-$ ,  $\text{Na}^+$ ,  $\text{Nd}^{+++}$ ,  $\text{Ni}^{++}$ ,  $\text{HPO}_4^{--}$ ,  $\text{Pb}^{++}$ ,  $\text{Pr}^{+++}$ ,  $\text{PtCl}_6^{--}$ ,  $\text{Rb}^+$ ,  $\text{ReO}_4^-$ ,  $\text{Rh}^{+++}$ ,  $\text{Ru}^{+++}$ ,  $\text{S}^{--}$ ,  $\text{SO}_4^{--}$ ,  $\text{Sb}^{+++}$ ,  $\text{Sc}^{+++}$ ,  $\text{SeO}_3^{--}$ ,  $\text{SiO}_3^{--}$ ,  $\text{Sm}^{+++}$ ,  $\text{Sr}^{++}$ ,  $\text{TaO}_4^{--}$ ,  $\text{TeO}_4^{--}$ ,  $\text{TiO}^{++}$ ,  $\text{Th}^{++++}$ ,  $\text{Ti}^{+++}$ ,  $\text{Tm}^{+++}$ ,  $\text{VO}^+$ ,  $\text{Y}^{+++}$ ,  $\text{Yb}^{+++}$ ,  $\text{Zn}^{++}$ ,  $\text{Zr}^{++++}$  ( $\text{ZrO}^{++}$ ).

Auric, ceric, and iridic ions produce brown precipitates with the reagent but these are extremely rare in tungsten ores and alloys.

Ferric iron reacts to form a small amount of brown precipitate but not enough to interfere even in the analysis of steels containing tungsten.

Stannous and stannic tins produce an orange and a yellow precipitate, respectively, with the reagent.

Copper reacts in alkaline solution to produce a brown precipitate but does not react at all in acid medium. In analysis, the reagent is always used in acid solution; hence copper presents no interference.

Molybdates are precipitated but not quantitatively. When molybdenum and tungsten are present together, a small amount of molybdenum may precipitate with the tungsten. The precipitation of molybdenum by the reagent is no greater than when cinchonine is used (see procedure for analysis of steels and alloys; cf. analysis of N.B.S. steel 132).

The only other ions found to react with the reagent are  $\text{UO}_2^{++}$  and  $\text{OsO}_5^{--}$ , which produce an orange and brown precipitate, respectively.

#### OPTIMUM EXPERIMENTAL CONDITIONS

**PERMISSIBLE ACIDITY.** Tungsten is quantitatively precipitated by the reagent when the acidity is less than pH 1. Partial precipitation occurs above pH 1 and above pH 5 no precipitation occurs. The best acidity to use is about 0.2N hydrochloric acid. Sulfuric and nitric acids may also be used, but in the presence of lead or tin sulfuric acid is objectionable and nitric acid solutions stronger than 1N begin to attack the reagent.

**TEMPERATURE OF SOLUTION.** Room temperature is preferable for effective precipitation of the tungsten complex. Precipitation begins immediately upon addition of the reagent in cold solution, with the precipitate collecting and settling much more readily than if the solution is hot. The reagent should therefore not be added to hot solutions.

**AMOUNT OF REAGENT REQUIRED.** The reagent combines with tungstate ions in a ratio of 1 to 1. However, when the approximate quantity of tungsten is known, it is recommended that twice the theoretical amount of reagent be added to ensure complete and rapid precipitation. If the tungsten content is unknown, the amount of reagent required may be judged by observing the color of the precipitated complex as it forms.

**TIME NECESSARY FOR COMPLETE PRECIPITATION.** Complete precipitation is obtained when solutions are allowed to stand at room temperature for 3 hours after the addition of excess reagent, with occasional stirring to collect the precipitate. Allowing solutions to stand overnight ensures complete precipitation but is not necessary.

**WASHING THE PRECIPITATE.** The most effective wash solution has been found to be a cold, dilute hydrochloric acid solution containing a small amount of the reagent. It is prepared by diluting 20 ml. of concentrated hydrochloric acid to 1 liter and adding 1 ml. of a saturated ethanol solution of the reagent.

**FINAL TREATMENT OF PRECIPITATE.** Attempts to weigh the organo-tungstate precipitate directly, after washing and drying,

indicate that the method is impractical. Conditions have to be tediously controlled to get consistent results. The difficulties arise from the fact that the reaction must occur in acid solution and that the reagent itself precipitates when added in small excess. Unless the reagent is added immediately after acidifying the solution, some tungsten may precipitate as tungstic acid and be occluded in the organic precipitate, preventing a consideration of it as a compound of definite composition. It is also difficult to remove precipitated reagent from the complex without at the same time dissolving some of the complex.

The complex may be ignited easily to tungstic oxide,  $\text{WO}_3$ , in which case it makes no difference if tungstic acid and excess reagent are present, because tungstic acid is converted to tungstic oxide and excess reagent is completely removed upon ignition.

The precipitated complex is easily and rapidly separated by filtration through quantitative ashless filter paper. Whatman No. 40 and other papers of similar texture and grade are suitable for quantitative analyses when using this reagent. Filtration is most rapidly accomplished if the precipitated complex is allowed to settle (about 3 hours is sufficient) and the solution filtered by decantation. There is very little tendency for the precipitate to clog the pores of the paper, thus permitting thorough washing with a minimum time consumption.

The washed precipitate may be ignited without difficulty. There is no tendency for explosion, or decomposition rapid enough to sweep out any of the contents of the crucible. The precipitate and the filter paper decompose at about the same rate. Moisture should be removed at about 100° C. before raising the temperature of the crucible and contents to ignition temperature. The crucible should not be heated above a dull red until all carbon has been burned off.

#### DETERMINATION OF TUNGSTEN IN SOLUTIONS, STEELS, ALLOYS, AND ORES

Of the various procedures investigated, a modification of that of Hillebrand and Lundell (6, pp. 553-5) has been found most satisfactory for the analysis of tungsten ores. For steels and tungsten alloys, modifications of the procedures of the American Society for Testing Materials (22, pp. 1011-2) are both speedy and accurate.

**DETERMINATION IN ORES.** Transfer to a 400-ml. beaker 1 gram of sample which has been ground in an agate mortar to 200-mesh or finer and dried to constant weight at 105° C. Unless the sample is ground to 200-mesh or finer, a protective layer of precipitated tungstic acid may coat the particles, preventing the hydrochloric and nitric acids from coming in contact with the unreacted material. This is especially true with ferberite and wolframite, which are difficult to decompose. If sufficient hydrochloric acid is used and the temperature is held at approximately 75° C., the formation of tungstic acid on the particles may be avoided. Serious error may be introduced by this effect if samples of particle size much larger than 200-mesh are used. The magnitude of the error depends on the mineral being analyzed. Scheelite,  $\text{CaWO}_4$ , and hubnerite,  $\text{MnWO}_4$ , are easily decomposed by the hydrochloric-nitric acid treatment, in which case the error is small; ferberite,  $\text{FeWO}_4$ , and wolframite,  $(\text{Fe,Mn})\text{WO}_4$ , being much more difficult to decompose, require very small particle size.

Add 5 ml. of distilled water and rotate the beaker so as to distribute the sample evenly over its bottom. Add 100 ml. of hydrochloric acid (sp. gr. 1.19), cover the beaker with a watch glass, and heat for one hour at a temperature not exceeding 60° C. Stir occasionally to break up formations of crusts and facilitate contact of the acid with all particles of the sample. Raise the watch glass on glass hooks and cautiously boil to volume of about 50 ml. Break up the material on the bottom of the beaker with a glass stirring rod. Add 40 ml. of hydrochloric acid and 15 ml. of nitric acid and again boil the solution to about 50 ml. Stir up the caked matter again, add 5 ml. of nitric acid and boil to 10 or 15 ml. Dilute to 250 ml. with hot water and heat just below boiling for 30 minutes. Allow to cool to room temperature and add 25 ml. of alcoholic reagent solution (made by dissolving 0.7 gram of reagent in 100 ml. of ethanol) slowly with constant stirring. Allow the precipitate to settle for about 2 hours and test the supernatant liquid with a few drops of the reagent for complete precipitation of the tungsten.



Because of the limited solubility of the reagent in aqueous solutions, some precipitate may be expected to form whenever the reagent is added. The appearance of the precipitate formed in the presence of tungsten is different from that formed when the latter is absent. The organic tungstate is deep yellow-orange in color while the precipitated reagent alone is almost white. It is advisable for an analyst using the reagent for the first time to add some of it to a solution of sodium or ammonium tungstate (mg. of tungstate per ml.) acidified with 8 or 10 volumes of 0.1N hydrochloric acid. The same amount of reagent should be added to a similar volume of 0.2N acid alone. The striking difference between the color of the reagent and that of the organo-tungstate may be observed by this simple procedure.

In testing a solution for completeness of precipitation of tungsten, more reagent should be added if an orange precipitate is formed. If it is white or pale yellow, precipitation of the tungsten is complete.

After the precipitate has settled at least 2 hours (or overnight if convenient) filter by decantation through an 11-cm. ashless filter paper. Wash the precipitate several times with reagent wash solution.

Transfer the precipitate to an ashless filter paper and moderately scrub the beaker by means of a rubber policeman to remove, as far as possible, any precipitate adhering to the walls of the beaker. It is not necessary at this point to attempt to remove the finely divided tungstic acid adhering to the walls of the beaker, since the main precipitate, after it is washed, is to be transferred back to this beaker and all the precipitated tungsten dissolved.

Wash with the prepared wash solution. Repeat several times and set aside the combined filtrate and washings. Test the filtrate with a few drops of reagent solution for complete precipitation of the tungsten. It is rare that any is found in this filtrate. Transfer the filter paper containing the washed precipitate to the original beaker and add 6 ml. of concentrated ammonium hydroxide. Shred the filter paper to a uniform pulp by means of a glass stirring rod, cover the beaker, and warm gently for a few minutes. Stir the pasty mass with the rod, then wash down the side of the beaker with warm dilute ammonium hydroxide (1 to 10 containing 10 grams of ammonium chloride per liter. Warm again and stir thoroughly. Filter through an 11-cm. ashless filter paper and collect the filtrate in a 400-ml. beaker. Wash the original beaker and residue several times with the warm dilute ammonium hydroxide solution; between washings, squeeze as much of the liquid from the fibers of the pulp as possible. Wash the beaker and residue with several small portions of hot 95 per cent ethanol to dissolve any organo-tungstate that has not been decomposed by the ammonium hydroxide treatment. Follow with a final washing with the warm dilute ammonium hydroxide solution. Keep the volume of liquid as small as possible. Reserve the residue of filter fiber for further recovery of traces of tungsten.

Evaporate the filtrate to a volume of about 50 ml.; add 20 ml. of concentrated hydrochloric acid and 10 ml. of concentrated nitric acid; cover and cautiously boil to a volume of 10 to 15 ml. Any organic residue is still present and tends to adhere to the walls of the beaker, it may be decomposed by the addition of more hydrochloric and nitric acids in the same ratio as above. Dilute the solution to about 250 ml. with water and allow it to cool slowly to room temperature. Add the alcoholic reagent solution until the color of the precipitate, as it forms, indicates complete precipitation of the tungsten. Allow the precipitate to settle and filter through an 11-cm. ashless filter paper; wash thoroughly with reagent wash solution. If the filtrate has a clear yellow tint, precipitation is complete. If it is colorless, more reagent must be added to complete the precipitation. The washed precipitate is the main precipitate and is to be ignited with the very small amounts of tungsten that may be obtained in the procedure described in the following paragraphs.

Any tungsten that may not have been recovered is contained in the reserved residue of filter fiber, in combination with iron and alumina or with small amounts of reagent that were not completely dissolved. This combined tungsten may be dissolved by digesting the filter paper and residue of fiber with warm dilute hydrochloric acid (1 to 9). Filter and wash the residue with small amounts of hot 0.5 per cent ammonium chloride solution and the warm dilute ammonium hydroxide wash solution, collecting all in the same vessel. Acidify the filtrate with hydrochloric acid until it is approximately 0.2N and then slowly add 5 to 10 ml. of alcoholic reagent solution. Any precipitate

obtained should be filtered and washed with the reagent wash solution. Reserve the washed precipitate and ignite later with the main one already obtained.

The residue that now remains is usually free from tungsten. To be positive, ignite it in a porcelain crucible (not a platinum crucible because tin might be present), transfer the ash to a platinum crucible, and volatilize the silicon by treating it with hydrofluoric and sulfuric acids. Fuse the remaining residue with as little sodium carbonate as possible, cool, and extract the melt with water. Filter and acidify the filtrate with hydrochloric acid, boil to expel carbon dioxide, and add dropwise some of the reagent solution to test for the possible presence of tungstate ions. If the precipitate is orange, upon the addition of the first few drops of reagent, filter it off, wash with the reagent wash solution and ignite with the two residues already obtained. (The authors have never obtained any organo-tungstate precipitate at this point.)

Place the papers containing the main precipitate and the two recoveries in a weighed platinum crucible and heat at a temperature below a dull red until all the carbon has been burned off. Cool, add a few drops of hydrofluoric acid to the residue (enough to moisten it completely), add a drop of sulfuric acid and evaporate to dryness over a water or sand bath. Reignite in order to get the weight of  $WO_3$  free from  $SiO_2$ .

The tungstic oxide obtained at this point is not pure but must be examined for contaminants. The examination for contaminants does not involve any changes from the standard procedures now in use (3; 6, p. 555; 22). The contaminants most apt to be present at this point are principally iron and molybdenum, with very small traces of phosphorus. The iron is separated by fusion with sodium carbonate, dissolving in hot water, and filtering. Tungsten and molybdenum (if present) form soluble sodium salts. If molybdenum is present, the amount of its contamination may be determined colorimetrically (3; 22, pp. 1008-9) in the filtrate. It is rare that the amount of phosphorus present justifies a test for it. The weights of contaminants found are subtracted as oxides from the weight of impure tungstic oxide and the corrected weight is used to calculate the percentage of tungstic oxide or tungsten in the sample.

**DETERMINATION IN ALLOYS AND METALS.** Treat 1 gram of the finely divided metallic sample with 5 ml. of hydrofluoric acid in a large covered platinum crucible or dish. After the initial effervescence has ceased, add nitric acid dropwise until the metal has dissolved. Add 15 ml. of sulfuric acid (1 to 1), transfer the vessel to a sand bath, and heat cautiously until dense fumes of sulfur trioxide are evolved freely. Perchloric acid may be substituted for sulfuric acid if desired. In this case, add 15 ml. of perchloric acid (60 per cent) after the hydrofluoric-nitric acid treatment and heat cautiously to dense fumes of perchloric acid. Cool, dilute, and transfer in the same manner as when sulfuric acid is used. The use of perchloric acid shortens slightly the time necessary for this part of the analysis, but otherwise offers no particular advantage.

Allow to cool and transfer the contents to a 400-ml. beaker by washing the platinum vessel with a fine stream of water. Wipe the vessel with a small piece of ashless filter paper and transfer it to the beaker. Rinse the vessel with a little warm ammonium hydroxide (1 to 1), a little water, then a little hot hydrochloric acid (1 to 1). Repeat the treatments with ammonium hydroxide, water, and hydrochloric acid, adding all rinsings to the 400-ml. beaker. Dilute the contents of the beaker with water to about 150 ml. Add 10 ml. of hydrochloric acid (sp. gr. 1.19), cover with a watch glass supported on glass hooks, and boil cautiously for at least 5 minutes. Remove the source of heat and dilute the contents to about 350 ml. with water. Allow to cool and add slowly, with constant stirring, 15 to 20 ml. of alcoholic reagent solution (0.7 gram per 100 ml. of 95 per cent ethanol). Allow the precipitate to settle for about 2 hours and test the supernatant liquid for complete precipitation of the tungsten. If an orange precipitate forms upon the addition of more reagent, precipitation is incomplete. If the precipitate is almost white, separation of tungsten is complete.

When the precipitate has completely settled, filter by decantation through an 11-cm. ashless paper. Wash the precipitate several times with reagent wash solution, ignite the paper and residue, in the platinum vessel in which the sample was treated originally, at a temperature below a dull red until all of the carbon is consumed. Add a few drops of nitric acid and evaporate to dryness on a water or sand bath. Ignite to constant weight (preferably in an electric muffle furnace) at a temperature not exceeding 750° C. This is the weight of impure tungstic oxide. Add about 5 grams of sodium carbonate and carefully heat until a clear melt is obtained. Rotate the fused mass in the vessel until it solidifies around the wall of the container. When cool, dissolve the melt in hot water, filter through an 11-cm. ashless paper, and wash thoroughly with hot water. Place the filter in



the crucible and ignite again. Repeat the sodium carbonate fusion on the small residue, using a proportionately smaller amount of carbonate than in the first fusion. Cool and dissolve in the same manner as before. Filter and wash thoroughly to remove all traces of sodium carbonate. Ignite in the same platinum vessel, cool, and weigh. Subtract the weight of this oxide residue from that of the original impure tungstic oxide. Calculate the percentage of tungsten from the corrected weight of tungstic oxide.

If molybdenum is present, the amount of its contamination of the tungsten precipitate can be determined in the sodium carbonate extracts. This is best done colorimetrically (3; 22, pp. 1008-9).

#### ANALYSIS OF STANDARD TUNGSTATE SOLUTIONS

A standard tungstate solution was prepared from C.P. tungstic acid ( $\text{H}_2\text{WO}_4$ , 99.84 per cent pure) by fusion with sodium hydroxide in a silver crucible. The fusion mass was dissolved in water and diluted to the mark in a volumetric flask. Measured volumes of this solution were taken and acidified to 0.2N with hydrochloric acid and the tungsten was precipitated by the addition of an ethanol solution of the new reagent. In order to compare the two reagents, the tungsten was also precipitated by cinchonine from a similar series of solutions. The precipitated complexes were filtered off through quantitative paper and ignited in platinum crucibles to tungstic oxide (Table I).

#### APPLICATION OF PROCEDURES

**ANALYSIS OF STEELS AND ALLOYS.** National Bureau of Standards samples Nos. 50a, 132, and 75 were used to study the applicability of the procedure to the determination of tungsten in steels and alloys (Table II). One-gram samples were used and the tungsten was precipitated and separated according to the procedure given for the analysis of tungsten alloys and metals.

**ANALYSIS OF ORES.** Samples of scheelite and ferberite (obtained through the courtesy of G. E. F. Lundell, National Bureau of Standards, Washington, D. C.), and wolframite (supplied by the Callite Tungsten Corporation, Union City, N. J.) were analyzed by the procedure outlined for the determination of tungsten in ores (Table III). One-gram samples were used.

#### DISCUSSION OF RESULTS OF ANALYSES

The results obtained in the analyses of the solutions, steels, alloys, and ores show that anti-1,5-di-(*p*-methoxyphenyl)-1-hydroxylamino-3-oximino-4-pentene may be applied to the gravi-

metric determination of tungsten in these materials. The variation of percentage values between individual samples is well within the range of variation encountered by the use of other acceptable gravimetric methods. With the National Bureau of Standards samples, the values found by this method are within the range of variation of values reported by the various analysts using other methods. The small variations in percentage found by this method are believed to be due to experimental technique rather than any inconsistency in the behavior of the new reagent.

In the analysis of N.B.S. steel 132, which contains approximately equal amounts of tungsten and molybdenum (7 per cent molybdenum and 6 per cent tungsten) it was necessary to determine the amount of molybdenum contaminating the tungsten precipitate and correct for it.

Table III. Ores

Sample	Material	WO <sub>3</sub> Present %	WO <sub>3</sub> Found %	Difference %
1	Scheelite	59.6 <sup>a</sup>	59.53	-0.1
2			59.70	+0.1
3	Ferberite	66.0 <sup>a</sup>	66.08	+0.1
4			66.25	+0.3
5			65.97	0.0
6	Wolframite	69.39 <sup>b</sup>	69.40	+0.01
7			69.23	-0.16

<sup>a</sup> Tentative values supplied by G. E. F. Lundell. Samples were dried for 18 hours at 105° C. before being analyzed by Dr. Lundell in 1918. They were dried under same conditions for analysis with new tungsten reagent after each was thoroughly mixed.

<sup>b</sup> Value supplied by Callite Tungsten Corporation on basis of air-dried sample of a commercial wolframite ore.

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Table I. Standard Tungsten Solutions

Sample	WO <sub>3</sub> Present Mg.	WO <sub>3</sub> Found by Cinchonine Mg.	WO <sub>3</sub> Found by New Reagent Mg.
1	9.1	9.1	9.1
2	18.5	18.5	18.5
3	18.0	17.9	18.0
4	18.0	18.1	18.1

Table II. Steels and Alloys

Sample	Material	W Present %	W Found %	Difference %
1	N.B.S. chrome-tungsten-vanadium steel No. 50a	18.25 <sup>a</sup>	18.35	+0.10
2			18.21	-0.04
3			18.27	+0.02
4	N.B.S. molybdenum-tungsten steel No. 132	6.29 <sup>a</sup>	6.58	+0.29 <sup>b</sup>
5			6.65	+0.36 <sup>b</sup>
6			6.73	+0.44 <sup>b</sup>
7			6.34	+0.05 <sup>c</sup>
8			6.34	+0.05 <sup>c</sup>
9	N.B.S. ferro-tungsten No. 75	75.2 <sup>a</sup>	75.36	+0.2
10			75.11	-0.1

<sup>a</sup> National Bureau of Standards certificate value.

<sup>b</sup> N.B.S. No. 132 contains 7% (approx.) molybdenum. In samples 4, 5, and 6, no correction was made on ignited tungstic oxide for molybdic oxide as a contaminant.

<sup>c</sup> In samples 7 and 8, molybdenum contaminating oxide was determined colorimetrically and corrections applied. High results of samples 4, 5, and 6 are evidently due to presence of molybdic oxide.



# Dichromate Determination of Iron, Using the Silver Reductor

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THE method of Walden, Hammett, and Edmonds (4) for the determination of iron using the silver reductor and titrating with ceric sulfate, although highly satisfactory, is expensive because of the relatively high cost of the ceric sulfate and its very high equivalent weight. In the titration large quantities of sulfuric acid are required. The dichromate method as presented in this paper overcomes these objections and seems to retain the more desirable features.

In the analysis of ores and alloys of iron the common impurities which may interfere with the reduction and subsequent titration are chromium, manganese, molybdenum, titanium, and vanadium. Molybdenum may be separated from iron by precipitating it as the hydrous oxide. In the silver reductor, chromium is not reduced below the trivalent state, while manganese and titanium are not reduced at all. Vanadium is reduced to the vanadyl ion which is not oxidized by either cerate or dichromate ions.

Table I. Effect of Added Impurities

(Volume of  $\text{FeCl}_3$ , 25 ml.)

Solution Added Ml.	No. of Detns.	Fe Found Gram	Average Deviation P.p. 1000
From previous standardization <sup>a</sup>	..	0.1755	..
One	6	0.1756	0.6
0.01M $\text{K}_2\text{Cr}_2\text{O}_7$	6	0.1757	1.1
0.1M $\text{MnCl}_2$	5	0.1760	2.8
0.1M $\text{Ti}(\text{SO}_4)_2$	10	0.1756	0.6
0.1M $\text{NaVO}_3$	2	0.1755	0
0.1M $\text{NaVO}_3$	2	0.1754	-0.6
0.1M $\text{NaVO}_3$	3	0.1754	-0.6
0.1M $\text{NaVO}_3$	2	0.1753	-1.1
10 ml. of each	2	0.1754	-0.6

<sup>a</sup> From the standard method of Walden, Hammett, and Edmonds.

The oxidation potential in 1N hydronium-ion concentration of the system,  $\text{VO}_4^{3-}-\text{VO}^{2+}$ , is given as 1.2 volts (1), and that of diphenylamine sulfonic acid is 0.83 volt (2), which according to Walden, Hammett, and Edmonds (3) is sufficiently below the vanadate-vanadyl potential to give precise results. Adjusting the acidity to approximately 1N and adding 5 ml. of 85% phosphoric acid, one obtains a condition favorable to the titration of ferrous iron with potassium dichromate even in the presence of vanadium.

## MATERIALS

Standard solutions of ceric ammonium sulfate and potassium dichromate were prepared in the usual manner and standardized against pure iron wire. They were also checked against a gravimetrically standardized solution of ferric chloride, using stannous chloride reduction.

A solution of ferric chloride standardized gravimetrically was used in studying the behavior of the dichromate-diphenylamine sulfonic acid titration in the presence of the ions of chromium, manganese, vanadium, and titanium.

The effect of impurities was studied by adding definite quantities of 0.01 molar potassium dichromate, 0.1 molar manganese chloride, 0.1 molar sodium vanadate, and 0.1 molar titanium sulfate. The latter was prepared by fusing 4 grams of titanium dioxide with 80 grams of potassium hydrogen sulfate, dissolving in 55 ml. of concentrated sulfuric acid, and diluting the resulting solution to 500 ml.

The silver reductor was prepared in the manner described by Walden, Hammett, and Edmonds (4).

## METHOD

Analyses were made with 25-ml. portions of the standard ferric chloride solution by the method of Walden, Hammett, and Edmonds (4), titrating with ceric ammonium sulfate. Similar analyses were then made by titrating with potassium dichromate. A 25-ml. sample of standard ferric chloride solution was pipetted, the acidity was adjusted to 1 molar with hydrochloric acid, and the final volume of 50 ml. was passed through the reductor

at a rate of about 30 ml. per minute. The reductor was washed with 150 ml. of 1 molar hydrochloric acid added in small portions. To the reduced solution 5 ml. of 85% phosphoric acid and 5 to 6 drops of 0.25% diphenylamine sulfonic acid were added, and the solution was titrated with potassium dichromate. The results, shown in Table I, deviated from those of the Walden method by only 0.06% error.

Similar analyses were made by introducing in the first series 25-ml. portions of 0.01 molar potassium dichromate, in the second 0.1 molar manganese chloride, and in the third 0.1 molar titanium sulfate. Controls were run on each ion by passing the solution through the reductor in the absence of iron. In each case one drop of 0.1 normal potassium dichromate gave a distinct end point.

It has been shown that reliable results can be obtained in the presence of chromium, manganese, or titanium with acidities ranging between 0.5 and 1.5 molar. Checks were obtained with titration acidities as low as 0.1 molar and as high as 2 molar, but at the extremes of concentration the end points were not sharp. The results shown in Table I indicate that these ions do not interfere with the analysis.

In the presence of high concentrations of vanadium an indistinct end point was obtained, the color change being from light green to gray. Titrations were made in the presence of a wide range of concentrations of vanadyl ion; up to concentrations of 100 mg. of vanadium per 200 ml. of titrating volume a very sharp end point was obtained. The deep violet color was not shown as in the absence of vanadium but there was a distinct color change from light green to deep blue. The results of these runs checked well with the accepted value for iron in the standard ferric chloride solution.

Two samples obtained from the Bureau of Standards were analyzed: iron ore, B. of S. No. 27, and ferrovanadium alloy, B. of S. No. 61. The samples were dissolved according to recommended procedures accompanying the samples. The removal of molybdenum from the alloy was accomplished by twice precipitating the hydrated ferric oxide from ammoniacal solution. In each instance the acidity was adjusted to 1 molar with hydrochloric acid and reduced as before. Results of the analyses are shown in Table II.

Table II. Determination of Iron

B. of S. Sample	No. of Detns.	Fe Found %	Fe, B. of S. Certificate %	Average Deviation P.p. 1000
Iron ore 27	4	69.30	69.26	0.6
Ferrovanadium alloy 61	2	52.83	52.8	...

## SUMMARY

Potassium dichromate, using diphenylamine sulfonic acid as indicator, is an oxidizing agent for the determination of iron by the Walden silver reductor method. Manganese, chromium, and titanium do not interfere. Vanadium does not interfere in concentrations of 100 mg. or less in 200 ml. of titrating solution.

A more economical method results from the use of only 1N hydrochloric acid instead of the higher concentrations of sulfuric acid required in the ceric sulfate method. The stability, purity, low equivalent weight, and comparatively low cost of potassium dichromate render it a very desirable oxidizing agent in this connection.

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# Precise Measurement of Volume in Titrimetric Analysis

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A review of precise measurement of volume in titrimetric analysis is presented. Detailed descriptions of the burets and special techniques used by the author are given. Representative standardizations indicate the reliability to be expected under the prescribed conditions.

ASIDE from the choice of a suitable chemical reaction, or the resultant of a series of successive reactions, on which to base a dependable titrimetric process, one is confronted with the responsibility of performing the physical measurements with sufficient nicety. More specifically, if the highest accuracy is to be attained, the following points must be given serious consideration: (1) influence of temperature change, (2) position of the meniscus, (3) drainage or afterflow, (4) errors of graduation, (5) evaporation, and (6) point of complete reaction.

Except for the last two, these sources of uncertainty can be removed by weighing, instead of measuring by volume, the standard solutions. For this purpose, various forms of "weight" or "weighing burets" have been invented; and a few investigators (1, 2, 15) have seen fit to take account of the solution of known concentration by weighing the reaction vessel both before and after the titration.

If the loss of water (or other solvent) from standard solutions during long periods of storage be left out of account, it is safe to assume that the evaporation taking place within the time required to effect a volumetric determination is of no significance (19). On the contrary, rendering a decision as to the equivalence point looms up as an unquestionably difficult objective in this kind of analysis, and one in which the weight buret, as compared with the volume buret, offers no advantage.

If the volume burets are properly designed, and used with certain precautions, a much higher degree of accuracy may be realized in dealing with a number of the well known volumetric solutions than is commonly supposed. The very fine work of Ponndorf (26) may be cited in support of the above contention, and this constitutes by no means the sum total of the evidence (cf. 11, 14, 21, 48).

Admittedly, when many titrations have to be made, it is expedient to feed the evaluated solution from a large reservoir directly into the buret. Moreover, in the case of solutions that are sensitive to the oxygen of the air and have to be kept under a nearly constant pressure of an indifferent gas (4, 17), the transfer to a short buret for weighing would probably lead to a lowering of the titer (28). Weighing the titration flask is not always feasible: in some experiments the reaction mixture must be heated; it is often desirable to bubble an inactive gas (carbon dioxide, for example) through the test solution (43); it is sometimes necessary to introduce an indicator during the latter stages of the procedure. However, Lee (18) has developed an ingenious weighing buret, wherein a known solution of titanous sulfate may be obtained by shaking acidulated titanous sulfate with zinc amalgam. But such a device, owing to the high density of the amalgam (about 4 per cent zinc, presumably), would seem to be unduly heavy, besides being restricted to the preparation of only a small quantity of the reducing agent at a time.

The chief limitation to measurement by volume in precise analysis is the lack of experimentally established data regarding the thermal expansion of numerous very useful solutions. This

expansiveness is a matter of some importance; hence its further study would seem to be justified.

## THERMAL EXPANSION

Thiessen and his collaborators, and also Chappuis, have determined the density of water ("ordinary water-substance") at various temperatures with great exactness. From their data the expansivity may be calculated. Accordingly, Circular 19 of the Bureau of Standards (5) enables one to correct the observed volume to what it would be at 20° C.—the standard temperature for volumetric analysis throughout the United States (24). The small numbers that are to be added or subtracted have been computed on the assumption that the glass forming the measuring utensils has a coefficient of cubical expansion of 0.000025 per degree Centigrade. (Certain borosilicate glasses, 47, such as Pyrex, exhibit thermal expansions much smaller than the foregoing.) These corrections apply not only to water but also, practically speaking, to sufficiently dilute aqueous solutions. Furthermore, a supplementary table (5, below Table 38) gives the percentage increase in the corrections for water to be applied when standard solutions of four common acids and bases—namely, nitric and sulfuric acids and sodium and potassium hydroxides—are under consideration. This increase is about 5 per cent for the before-named reagents when of 0.1N concentration. In like manner, it is but 3 per cent for 0.1N hydrochloric acid (44)—an almost negligible increment. Yet the higher the normality of the solution the greater must be the augmentation of the temperature corrections for pure water.

As early as 1869, Gerlach (13) studied the expansion of aqueous solutions of acids and salts; and in 1877 Casamajor (6), utilizing Matthiessen's data for water, attempted to correct the volume of his standard solution for changes of temperature. Some 5 years later, Schulze (39) determined the rate of expansion for a good many of the better known volumetric solutions, and these apparently reliable values have been used to a considerable extent. Schloesser (30, 31) and several other authorities (7, 9, 10, 20, 25, 38) have contributed to the subject in one way or another. Finally, Osaka (22) has made available his extensive investigations.

In this connection, it may be desirable to call attention to a point that is apt to be overlooked. When an auxiliary reagent is added to a titrimetric solution, even though it does not enter into the stoichiometric relations, it must needs alter the thermal expansion of the liquid. Many such cases might be cited, but a

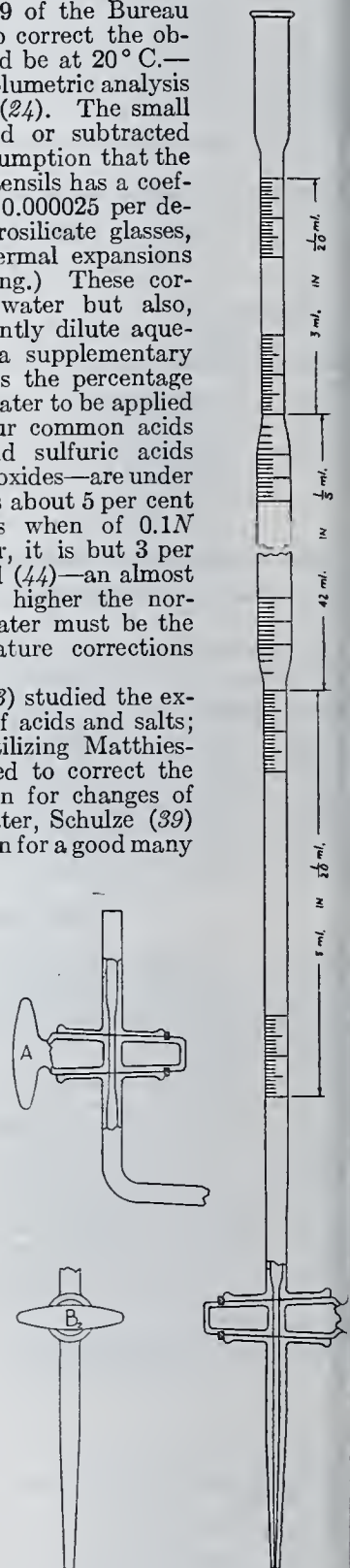


Figure 1



will suffice. Addition of potassium iodide increases the solubility of iodine, acidification of ammonium sulfato-cerate prevents hydrolytic decomposition of the cerium compound, and so on. In all candor, there seems to be a dearth of information concerning the expansivity of the more complex volumetric solutions.

Besides dilatation or contraction, certain other physical properties of an aqueous solution—namely, density, surface tension, and viscosity (37)—undergo appreciable change when the concentration with respect to a dissolved substance is markedly increased. As these properties influence delivery, the usual assumption that the volume measured at a given temperature is equal to that of pure water may not be fully warranted in the case of the complex solutions discussed above.

#### BURETS

The gratifying results obtained by Getz and the writer (42) in standardizing 0.1N barium hydroxide (measured from an ordinary buret) against benzoic acid and *p*-nitrobenzoic acid, respectively, led to the expectation that, with a special instrument of comparatively simple construction, it might be possible to attain a very high degree of precision in certain titrimetric analyses.

Two such burets (Figure 1) were procured. They were made by Eimer and Amend, of New York City, in accordance with definite specifications. Each instrument consists essentially of a middle wider portion, graduated in intervals of 0.2 ml., and two narrower portions, one above and one below, both marked in intervals of 0.05 ml. The total capacity is 50 ml., and the entire graduated length is approximately 70 cm. Each stopcock, which is somewhat larger than usual, is composed of a blown (and therefore hollow) plug and a neatly fitting shell. The former turns in the latter with the utmost smoothness. This low-friction bearing and the long handle (more than 5 cm.) permit the operator to release fractions of drops when nearing the end point. These stopcocks do not leak at all.

The following statements and recommendations regarding this type of buret are held to be self-evident. No matter what may be the magnitude of the sample, a reading will be obtained in any titration, provided the required volume does not exceed 50 ml., for the entire interval is graduated—even the shoulders. However, the level of the liquid stands between the 3- and the 45-ml. marks, the determination should be taken as tentative only. But, with this datum in hand, it is a simple problem to calculate by means of a proportion what increase in the weight of the sample will give rise to a reading somewhere between the 3- and the 45-ml. graduations, where, on grounds of probability, the best observations are to be had. (The linear value corresponding to 1 ml. is about 34 mm. in the narrower portions of the buret.)

In order to measure volumes somewhat less than 45 ml., a 50-ml. Normax buret was provided. This instrument is fashioned from a straight tube, the smallest division is 0.1 ml., and the distance representing 1 ml. is 16.3 mm.

To make the burets ready for use, a uniform, thin layer of a suitable lubricant is spread between the plug and the shell of the stopcock. With the plug in position *B*<sub>2</sub>, Figure 1, a small amount of carbon tetrachloride is poured into the buret. The key is then turned through an angle of 90°, *B*<sub>1</sub>, and the issuing liquid is caught in a small beaker. The jet being momentarily closed by the index finger, the same portion of the tetrachloride is again introduced into the buret at its top opening; but on no account should the key be turned back to position *B*<sub>2</sub> at this juncture, for fear that a furrow will be cut in the film of lubricant by the solvent. These operations are repeated until the small tubes have been washed free from visible greasy matter; whereupon the buret is dried thoroughly by aspiration.

The well known "cleaning mixture" (concentrated sulfuric acid to which powdered potassium dichromate has been added in abundance) is then applied, the preceding instructions as to the two positions of the stopcock key (more particularly, the plug capillary) being adhered to in an equally conscientious manner.

In calibrating the burets, the gravimetric method of Peffer and Mulligan (24) was followed in a general way. However, great care was taken to discharge the water very slowly—not faster than by rapid dropping from the tip, even during the initial stages of the delivery—and the settings and removal of hanging drops were made with all necessary pains.

Experience has shown that very uniform volumes of pure water may be obtained by slow delivery from a scrupulously clean

buret. This observation is in substantial agreement with the words of Osborne and Veazey (23): "By limiting the rate of outflow the residue and the afterflow may be made negligibly small."

The results obtained in calibrating the three burets are given in Table I. Judging from duplicate determinations, it is reasonable to hope that, in the case of the special burets (Eimer and Amend 12 and 13, respectively), measurements will agree to within 0.005 ml., and with the 25-ml. buret (Normax 830), to within 0.01 ml.

Table I. Calibration of Burets

(Volumes corrected to 20° C.)

Interval Ml.	Delivery Ml.	Rounded Value Ml.
E. and A. Buret No. 12		
0- 3	2.996	2.995 <sup>a</sup>
	2.995	
0-25	25.00	25.00 <sup>b</sup>
0-45	45.086	45.085 <sup>a</sup>
	45.086	
0-47.5	47.606	47.605 <sup>a</sup>
	47.608	
0-50	50.080	50.080 <sup>a</sup>
	50.082	
E. and A. Buret No. 13		
0- 2.5	2.517	2.515 <sup>a</sup>
	2.514	
0-25	24.84	24.84 <sup>b</sup>
0-45	45.068	45.070 <sup>a</sup>
	45.068	
0-47.5	47.579	47.580 <sup>a</sup>
	47.580	
0-50	50.068	50.070 <sup>a</sup>
	50.067	
Normax Buret No. 830		
0- 5	4.992	4.99 <sup>b</sup>
	4.994	
0-10	9.995	9.99 <sup>b</sup>
	9.994	
0-15	14.998	15.00 <sup>b</sup>
	14.996	
0-20	19.999	20.00 <sup>b</sup>
	19.999	
0-25	24.997	25.00 <sup>b</sup>
	25.006	

<sup>a</sup> Mean value rounded off to nearest 0.005 ml.

<sup>b</sup> Mean value rounded off to nearest 0.01 ml.

#### STANDARDIZATION OF SOLUTIONS

By way of forming a fairly satisfactory estimate as to the precision of the physical measurements in really nice volumetric analysis, wherein the titrations are made with volume burets, three familiar titrimetric solutions, of 0.1N strength, were standardized in accordance with supposedly accurate methods. The solutions were: (1) potassium permanganate, (2) sodium tetraborate, or borax, and (3) sodium hydroxide.

In the first set of experiments, 0.1N solutions of potassium permanganate were evaluated against the certified sodium oxalate of the National Bureau of Standards (Standard Sample 40c), the procedure of Fowler and Bright (12) being followed in all its minutiae.

The solutions were prepared by dissolving high-grade crystals of potassium permanganate in the requisite quantity of distilled water, aging for more than a month, and filtering very slowly through glass frit of fine texture under the influence of mild suction.

The clear solution was introduced into the buret by means of the "lift" (Figure 2), an apparatus modeled after a similar contrivance by Osborne and Veazey (23). The suction being turned on and properly regulated by obvious manipulations of the stopcocks, *S* and *T*, any desired quantity of the liquid may be pulled up from the storage bottle into a large pipet, *P*, held there at will, and finally allowed to enter the buret. Transferring in this manner leaves the neck of the bottle clean—a condition to be wished for in the case of a permanganate solution.

The temperature of the solution in the buret was taken by inserting a slender thermometer with enclosed scale, just before setting the meniscus upon the 0-ml. graduation, and again at the end of the titration, by running the remaining liquid into a



Table II. Potassium Permanganate against Sodium Oxalate

Solution No.	Date	Na <sub>2</sub> C <sub>2</sub> O <sub>4</sub> Gram	KMnO <sub>4</sub> Ml.	Normality
I	June 25	0.311 55	45.325	0.10260
	June 26	0.312 15	45.435	0.10255
	June 27	0.313 15	45.570	0.10257
	July 8	0.310 85	45.225	0.10259
	July 9	0.311 45	45.320	0.10258
			Mean	0.10258
II	May 24	0.316 65	47.420	0.09967
	May 24	0.315 60	47.270	0.09966
	Aug. 5	0.316 85	47.450	0.09967
	Aug. 5	0.316 60	47.415	0.09966
			Mean	0.09966
III	Aug. 16	0.317 05	47.600	0.09940
	Aug. 19	0.317 85	47.725	0.09939
			Mean	0.09940
IV	Jan. 18	0.317 05	47.570	0.09947
	Jan. 20	0.317 90	47.675	0.09951
			Mean	0.09949

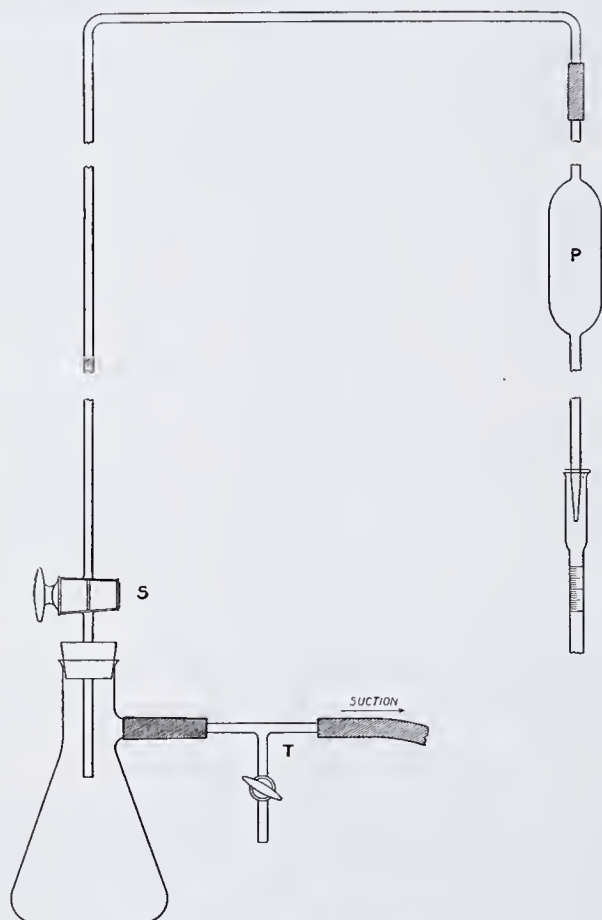


Figure 2. Lift Apparatus

small test tube. The mean of the two values was arbitrarily accepted, and the volume was adjusted on the assumption that the thermal expansion is the same as that of pure water (22).

Excellent observations of the meniscus were made by utilizing a device similar to the one proposed by White (46), despite the purple color of the solution.

The tiny excess of permanganate unavoidably added was estimated by matching the pink color of the test solution in the usual way. But it is doubtless more accurate to arrive at the correction iodometrically, as suggested by Bray (3).

The results obtained with potassium permanganate are set forth in Table II.

In the second set of experiments, the primary standard was constant-boiling hydrochloric acid (41), and from this a 0.1N acid was obtained. A solution of sodium tetraborate, likewise 0.1N, was prepared from recrystallized borax (16). For making the standardizations, the borax solution was weighed in a stoppered flask and was then titrated with the hydrochloric acid (in buret 12), methyl red serving as indicator.

A slight lack of uniformity in the results was apparent, owing presumably, to an end point that is somewhat difficult to judge and, because of this uncertainty, the tests with borax are not tabulated in the present report. Nevertheless, as the average deviation of a determination was less than 3 parts per 10,000 this part of the work may still be regarded as acceptable.

In the third set of experiments, a 0.1N solution of sodium hydroxide was prepared, reasonably free from carbonate, by the method of Cowles (8); it was protected against atmospheric carbon dioxide in the usual manner. A second basic solution, all respects similar to the first, was also employed. The sodium hydroxide was standardized against 0.2N hydrochloric acid, which, in its turn, had been obtained by appropriately diluting constant-boiling acid.

For alkaline Solution I (see Table III), a reference color was established by adding 1 drop of methyl red (0.2 per cent alcohol solution) to 100 ml. of water. For Solution II, 25 ml. of 0.2 sodium chloride were diluted to 100 ml., and the before-named quantity of the indicator was introduced. The latter mixture is theoretically correct, but it differs very little from the former in tint.

Twenty-five milliliters of the 0.2N hydrochloric acid were measured from the small buret (Normax 830), 25 ml. of water and a single drop of the methyl red were added, and the titration was performed with the 0.1N sodium hydroxide (in buret 12) the standard color being matched as well as possible.

The results of this last series of experiments, wherein all titrations were measured by volume, are given in Table III.

Table III. Sodium Hydroxide against Hydrochloric Acid

Solution No.	Date	0.20066 N HCl Ml.	NaOH Ml.	Normality
I	July 19	24.95	47.525	0.10534
	July 19	24.94	47.500	0.10536
			Mean	0.10535
II	Oct. 13	24.98	47.165	0.10628
	Oct. 13	24.98	47.145	0.10632
	Oct. 14	24.98	47.145	0.10632
			Mean	0.10631

## CONCLUSIONS

Barring such remarkably accurate results as those attained in iodometry by Washburn (45), who used a weight buret of the Ripper type (27), the experimental data reported herein compare favorably with many to be found in the scientific literature which were obtained by weighing, and not by measuring the volume of, the standard solution.

The delivery of a liquid from a graduated tube is at best somewhat empirical operation, and, naturally, the best measurements may be expected when the physical properties of the solution under consideration are near to those of the liquid (usually water) employed in calibrating the instrument. Moreover, especially, the thermal expansion of the titrant should be known at least approximately. These and many other points have been discussed and elucidated by such prominent metrologists as Schloesser (29-37), Osborne and Veazey (23), and Stott (40), whose writings the interested reader is referred to for further information and advice.

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# Ethylbis-2,4-Dinitrophenylacetate, a New pH Indicator

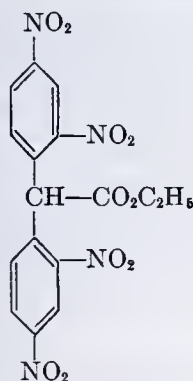
## Determination of Saponification Equivalents in Dark-Colored Oils

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A new acid-base indicator, ethylbis-2,4-dinitrophenylacetate, has been studied and its preparation described. The pH range over which the change from colorless to deep blue occurs is found to be from 7.5 to 9.1 (pK ca. 8.3), making the indicator suitable for most titrations which are ordinarily performed with phenolphthalein. The indicator gives an accurate end point in amber-colored solutions where the phenolphthalein end point is not visible, and it is therefore recommended for use in the determination of acid numbers and saponification equivalents of dark-colored oils.

IN THE course of an investigation on the preparation and properties of substituted phenylmalonic esters, the compound identified by Richter (6) as ethylbis-2,4-dinitrophenylacetate was obtained, and the opportunity was taken to study



suited to the titration of orange- and red-colored solutions in which the phenolphthalein end point is not visible. Because of the frequency with which dark-colored oils are encountered in organic analysis and because of the inadequacy of present-day methods of performing titrations with these products (1-5), data were obtained to determine the applicability of this indicator for such work. It was found that satisfactory results are obtainable even with extremely dark-colored oils and that the accuracy of the method approaches that of ordinary phenolphthalein titrations.

### PREPARATION AND PROPERTIES

The indicator is not difficult to prepare and, while the yield is not good, enough of the compound may be obtained from 0.5 mole of dinitrochlorobenzene to serve for thousands of titrations. The following procedure furnished 19.0 grams (18.1 per cent of theoretical) of recrystallized material sufficiently pure for use as an indicator.

One-half gram-atom (11.5 grams) of sodium was dissolved in 200 ml. of absolute alcohol in a 1-liter three-necked flask fitted with a reflux condenser, motor stirrer, and dropping funnel. The solution was cooled and 0.25 mole of diethyl malonate was added dropwise, with stirring, over a 30-minute period. Stirring was continued for another 10 minutes and a hot solution of 0.5 mole of 2,4-dinitrochlorobenzene in about 200 ml. of absolute alcohol was then added over a 30-minute period. The deep red reaction

indicator properties. Richter noted the color change of this compound from colorless in acid to intense blue in base and accordingly suggested its possible application as an acid-base indicator, but he reported no further studies along this line. The authors' results show not only that the compound may serve as a satisfactory substitute for phenolphthalein in the titration of most weak acids with strong bases, but also that it is specially



mixture was refluxed, with stirring, for 4 hours and allowed to stand overnight. Enough water was then added to the resulting olive-brown solution to bring the volume up to about 1200 ml., after which the solution was acidified with a little concentrated sulfuric acid, stirred for 20 minutes, then allowed to stand for 30 minutes. The water layer was decanted and the residual tar washed twice with water and finally with successive 200-ml. portions of alcohol until a granular black mass was obtained. By repeated washing with hot alcohol an orange solid was finally obtained, which, after recrystallization from benzene, gave pale yellow crystals melting at 150–153.5° C. (uncorrected).

(A small amount of a second compound with indicator properties can be isolated from the alcohol washings; it appears to be the monophenylated ester, ethyl-2,4-dinitrophenylmalonate, which would be expected as an intermediate in the formation of the diphenylated ester. After recrystallization from alcohol this compound melted at 48° C. and dissolved in dilute base to give a deep red color. The change from colorless to red was found to occur over a pH range of ca. 7.2 to 9.0, but the compound is not recommended as a substitute for phenolphthalein because the change is less distinct.)

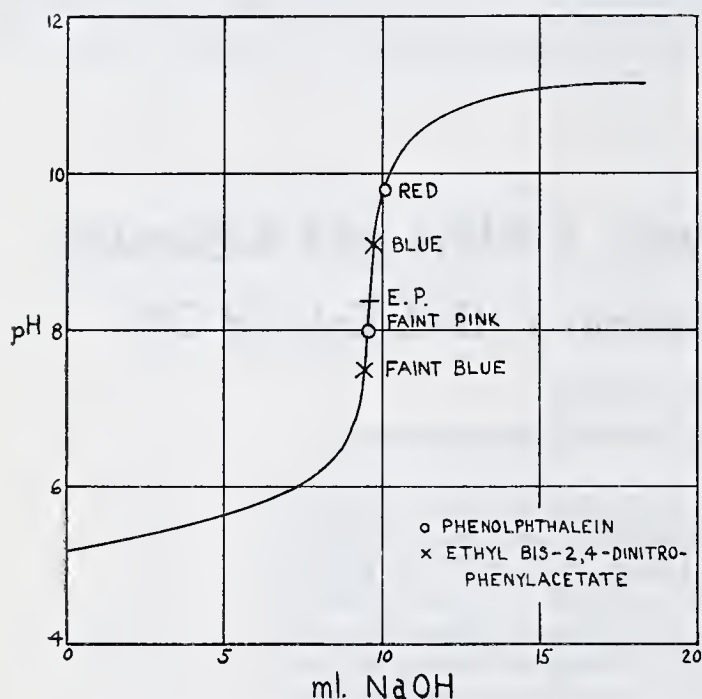


Figure 1. Indicator Ranges in Titration of Potassium Acid Phthalate with Sodium Hydroxide

The exact range over which the color change occurs was determined by titration of a buffered hydrochloric acid solution with sodium hydroxide, using the glass electrode to measure pH. About 5 drops of a saturated solution of the indicator in 50–50 acetone–ethyl alcohol were added to each 100 ml. of solution to be titrated. At pH 7.5 the first blue tinge appeared in the solution, and at pH 9.1 the change to deep blue was complete. (The indicator has been successfully used in the authors' analytical laboratories by students who, by reason of deficiencies in color vision, do not easily recognize the phenolphthalein end point.)

The pK of the indicator is therefore about 8.3. Figure 1 shows the indicator range given by ethylbis-2,4-dinitrophenylacetate compared with that given by phenolphthalein in the titration of potassium acid phthalate with sodium hydroxide. The same reversible color change was observed to occur in anhydrous solvents such as absolute alcohol, dry ether, and dry benzene.

#### TITRATION OF DARK-COLORED OILS

Since the basic color of ethylbis-2,4-dinitrophenylacetate, instead of being masked in amber-colored solutions as is the case with phenolphthalein, becomes more pronounced as a result of the complementary spectral relations, the end point is easily visible even in titrations involving dark-colored oils. In order to check the accuracy of the end point determined under

Table I. Saponification Equivalents Determined for Colored Oils (Using ethylbis-2,4-dinitrophenylacetate as indicator)

Oil	Saponification Equivalent		Approximate Color Density
	Calculated	Observed Un-colored Colored	
Propyl benzoate	164	165	1.85
Propyl benzoate	164	167	1.85
Propyl benzoate	164	166	1.85
Ethyl-3-carbethoxy-2-furanacetate	113	...	1.85
Ethyl-3-carbethoxy-2-furanacetate	113	...	1.85
Oil A	...	...	1.55
Oil A	...	...	1.55
Oil A (electrometric)	...	...	1.55
Oil B	...	...	60.3
Oil B	...	...	60.3

these conditions, saponification equivalents were determined for samples of pure propyl benzoate and ethyl-3-carbethoxy-2-furanacetate both before and after the addition of an artificial coloring material. For this purpose the addition of a few milliliters of a highly colored neutral caramel solution prepared from pure cane sugar was found to be satisfactory.

About 0.3 gram of the carefully distilled ester was weighed into a 125-ml. Erlenmeyer flask and the mixture was saponified with 15.00 ml. of a standard alcoholic sodium hydroxide solution according to the procedure of Shriner and Fuson (7). The saponification mixture was titrated almost to neutrality with standard acid, 5 drops of the indicator solution were added, and the end point was determined by adding an excess of acid and back-titrating with standard alkali to the first blue tinge. Several milliliters of aqueous caramel were then added to the alkaline solution and the end point was redetermined in the dark green mixture by adding acid until the amber caramel color just returned. In the dark-colored solutions the end point was approached from the basic side, not from the acid side as in the case of the uncolored solutions.

In Table I the saponification equivalents thus determined for uncolored propyl benzoate and for artificially colored propyl benzoate and ethyl-3-carbethoxy-2-furanacetate are compared with the true values calculated from the molecular weights of the esters. The agreement between the calculated and the observed values is sufficiently good to prove the usefulness of the procedure as an analytical method.

Two heat-bodied linseed oils, one of which (oil B, Table I) was almost opaque when viewed by daylight in thicknesses greater than 5 cm., were studied in order to determine the reproducibility of the results under actual working conditions. Oil A was also subjected to electrometric titration, thus affording an additional check on the accuracy of the results. The following procedure is recommended as a working method.

A sample of 0.2 to 0.4 gram of the oil was saponified as before and after reducing the alkalinity of the mixture by titrating almost to neutrality with 0.25*N* hydrochloric acid, 5 drops of a saturated solution of the indicator were added. More acid was then run in until the dark green color of the indicator just disappeared. Two more determinations of the end point were then carried out on the same sample by adding a few milliliters of standard sodium hydroxide solution and back-titrating with acid. The results of the triplicate determination were then averaged and the saponification equivalent was calculated. The electrometric titration on a saponified sample of oil A was performed in the usual manner, using a Beckman pH meter and a glass electrode.

As can be seen by reference to Table I, the values for oil B obtained by means of the relatively simple indicator method agree with the value obtained from the more elaborate electrometric method, and even the very high color density of oil B does not seriously interfere with the precision of the determination.

The approximate density of the amber color of both the naturally and the artificially colored oils is recorded in the last column of Table I with reference to 0.017*M* potassium dichromate solution arbitrarily assigned a value of unity. The



Light absorption was measured by comparison of the various oils with the standard on a Klett-Summerson photoelectric colorimeter without a filter. With the instrument adjusted to give a scale reading,  $R$ , of zero for the reference standard, the ratio of the light absorbed by the oil,  $i_x$ , to that absorbed by the standard,  $i_s$ , was calculated from the equation

$$\log \frac{i_x}{i_s} = 0.002R$$

$$\frac{i_x}{i_s} = \text{antilog } 0.002R$$

Thus oil B has a light-absorbing power approximately 60 times that of 0.017*M* potassium dichromate solution.

#### ACKNOWLEDGMENT

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## Pycnometer for Volatile Liquids

### Control of Diffusion as an Aid in Precision Pycnometry

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This paper reports the design of a pycnometer which is especially well suited for the determination of density on 5 ml. of volatile liquid with an accuracy of  $\pm 0.0001$  gram per ml.

FURTHER publication in the field of density determinations might seem unnecessary in view of the large number of devices which have already been described. However, most of these instruments were designed for a special purpose and a simple pycnometer for general use in hydrocarbon analysis, and particularly for obtaining precise densities on materials as volatile as pentane, has not previously been described.

Many general references (1, 9, 15, 19, 20, 24, 29) are available on the determination of density, and Irving (16) gives a general discussion of the method of floating equilibrium. Unusual methods include measuring the frequency of acoustic vibration (17) which is dependent on the density of the surrounding medium. Especially interesting are the balanced column methods (4, 7, 11, 23) which eliminate the use of an analytical balance, and which merit further investigation.

The more conventional pycnometers, such as the pipet types (3, 10, 33) and the Sprengel-Ostwald type with its modifications (3, 19, 20, 23, 27), often involve some kind of closure or cap to prevent rapid vaporization of volatile materials. These caps are in part successful, but are not entirely satisfactory with materials as volatile as pentane and ether.

In 1884 Perkin (22) recognized the advantage of having both menisci remote from the ends of the pycnometer arms, so that vaporization would be somewhat hindered. This principle is involved in the design of several flask-type pycnometers (2, 3, 30, 31) and many capillary-arm pycnometers (5, 12, 21, 22, 25, 26). However, the control of diffusion by the use of an unfilled capillary arm has not been generally recognized and has never, as far as the authors are aware, been discussed on a quantitative basis.

#### PYCNOMETER

The pycnometer which has been developed (Figure 1) has been in use in these laboratories for about 4 years. It is of Pyrex and consists of a 0.3-, 1-, 3-, or 5-ml. bulb blown in one side arm of a capillary U-tube. About 2 cm. of the upper end of the other side arm are bent over to form a hook for filling the pycnometer by capillary action and for hanging the pycnometer in the balance.

This hook is a self-filling device, previously described by Henion (14). The liquid is first drawn into the pycnometer by capillary action and the pycnometer then fills by siphoning.

Parker and Parker (21) also used the principle of siphoning to fill their pycnometer. Siphoning has the definite advantage over filling by a suction technique that it reduces the loss of the more volatile components of gasoline.

Both upper side arms of the U-tube are calibrated in scale divisions from 0 to 8.0 with ten intermediate scale divisions between each major scale division. Each major scale division equals 10 mm. The capillary tube should have a 0.6- to 1.0-mm. bore and should not be over 6 mm. in outside diameter. The pycnometer is similar to those of Shedlovsky and Brown (26) and Robertson (25), which feature two calibrated stems and 25-ml. capacity. The present pycnometer, however, is of much smaller capacity and is easily handled on an analytical balance. It is constructed with a bulb in only one side arm, which allows the pycnometer to be filled easily and avoids loss of volatile products by the slow rate of diffusion through unfilled capillary arms.

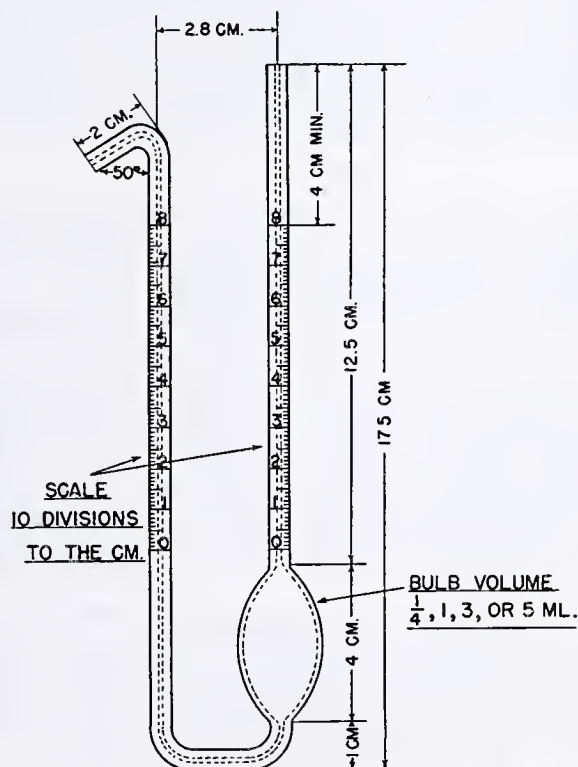


Figure 1. Pycnometer

Capillary bore 0.6 to 1.0 mm., outside diameter 6 mm. maximum, material Pyrex, total weight not to exceed 30 grams

<sup>1</sup> Present address, U. S. Rubber Company, Passaic, N. J.



The data showing the restraining effect of diffusion through a capillary on rate of evaporation, summarized in Figure 2, were obtained using a preliminary design in which both arms of the pycnometer were straight. Recent tests with the pycnometer having one arm bent are similar. Total length referred to is the sum of the unfilled capillary in both arms of the pycnometer and the rate of evaporation is the total rate from both arms of the pycnometer in milliliters per minute. The rates given in Figure 2 are based upon data obtained during July and August in a hot, drafty laboratory. Therefore these rates are probably maximum since heat and drafts on the exposed ends of the pycnometer both increase vaporization. However, these rates are sufficiently accurate to establish the length of the unfilled capillary necessary to avoid volatilization. Under more favorable conditions the evaporation rates are somewhat lower than shown in Figure 2.

It is clear from Figure 2 that if total length of unfilled capillary is over 10 cm., the rate of diffusion is so low that the vapor loss from the pycnometer becomes negligible.

The specification for these pycnometers is 0.6- to 1.0-mm. inside capillary diameter. Since the data were obtained on a pycnometer of 1.0-mm. capillary, the evaporation losses will not be greater than indicated in Figure 2.

#### CALIBRATION OF PYCNOMETER

The pycnometer calibration is based on the density of water and is checked with a pure hydrocarbon such as benzene. [Benzene may be easily purified by the following procedure: Place 5 gallons of commercial c.p. benzene in a can surrounded with 5 cm. (2 inches) of felt, and place the can in a cold room at about  $-30^{\circ}\text{C}$ . ( $-22^{\circ}\text{F}$ .), or if this is not available, in a box chilled with dry ice. Allow the benzene to freeze without agitation until only about 1000 cc. of benzene remain uncrystallized. Pour off the impure benzene, melt the crystallized benzene and recrystallize. Each crystallization should take 3 to 5 days. Five or six such slow crystallizations will usually give benzene having a freezing point of  $5.51^{\circ}\text{C}$ . (31), and density and refractive index agreeing with the best literature values.]

The volume at  $20^{\circ}\text{C}$ . of water free from air (boiled and cooled without agitation shortly before use) is obtained near the top, bottom, and middle of the scales of the pycnometer. The volume at  $20^{\circ}\text{C}$ . is calculated by dividing the weight of water by 0.99823. A calibration curve is drawn, plotting the sum of the scale divisions on both arms as the abscissa and the volume in milliliters as the ordinate. All points must fall on the same straight line, which is the calibration curve for the pycnometer. No correction is made for air buoyancy in the water calibration. Instead, a correction,  $C$ , given by Equation 1, is added to the observed value of the density to take care of all buoyancy errors in which  $D_0$  = density in air. This correction

$$C = 0.0012 \times (1 - D_0) \quad (1)$$

is based on the use of an average value of 0.0012 gram per ml. for the density of air (29), and is applicable to all types of weights, provided that weights of the same density are used in both the calibration and the density determinations.

The use of a sealed counterpoise has not been recommended, since the total volume of the 5-ml. pycnometer is only about 15 ml. and a variation in air density of  $\pm 0.00005$  gram per ml. will give a change in buoyancy of only 0.00075 gram. Since about 4 grams of liquid are weighed, this buoyancy variation corresponds to about  $\pm 0.00015$  in the density of the sample. This value is probably extreme. For more accurate work, especially with pycnometers of larger size, a sealed counterpoise can be used to compensate accurately for buoyancy of the air on the pycnometer itself. The buoyancy effect can then be corrected using an accurate value for the density of air, which is dependent on the temperature and humidity.

Check points on the water calibration are obtained with benzene. To obtain volumes, the weights of benzene are divided by 0.8788. The density of benzene in vacuum at  $20^{\circ}\text{C}$ . determined by use of this calibration curve and Equation 1 should be 0.87893, which checks the values of 0.87890 of Timmermans and Martin (28) and 0.87895 of Wojciechowski (32).

#### DETERMINATION OF PRECISION DENSITIES

A. PURE COMPOUNDS AND MIXTURES OF MEDIUM AND LOW VOLATILITY. The pycnometer is cleaned with benzene and

dried by suction. The final rinse should be with good clean benzene. Acetone is not recommended because it frequently contains nonvolatile residue. If the outside of the pycnometer is dirty or oily, it is rinsed with benzene and dried by gently wiping with cheese cloth or other suitable material. [Static charge introduced by wiping the pycnometer with a dry cloth is apt to cause errors in weighing, especially in cold dry weather. Before hanging pycnometer on wire hook in the balance, observe whether pycnometer exerts attraction for the wire hook by touching the hook with the pycnometer and gently drawing it away. Static charge can usually be nullified by touching the side of the pycnometer lightly with one's fingers. If this procedure will not remove the static charge, the humidity of the balance room should be increased until the difficulty disappears (about 60 percent relative humidity).] The weight is then obtained within  $\pm 0.0001$  gram on a good analytical balance, after allowing the pycnometer to come to balance case temperature.

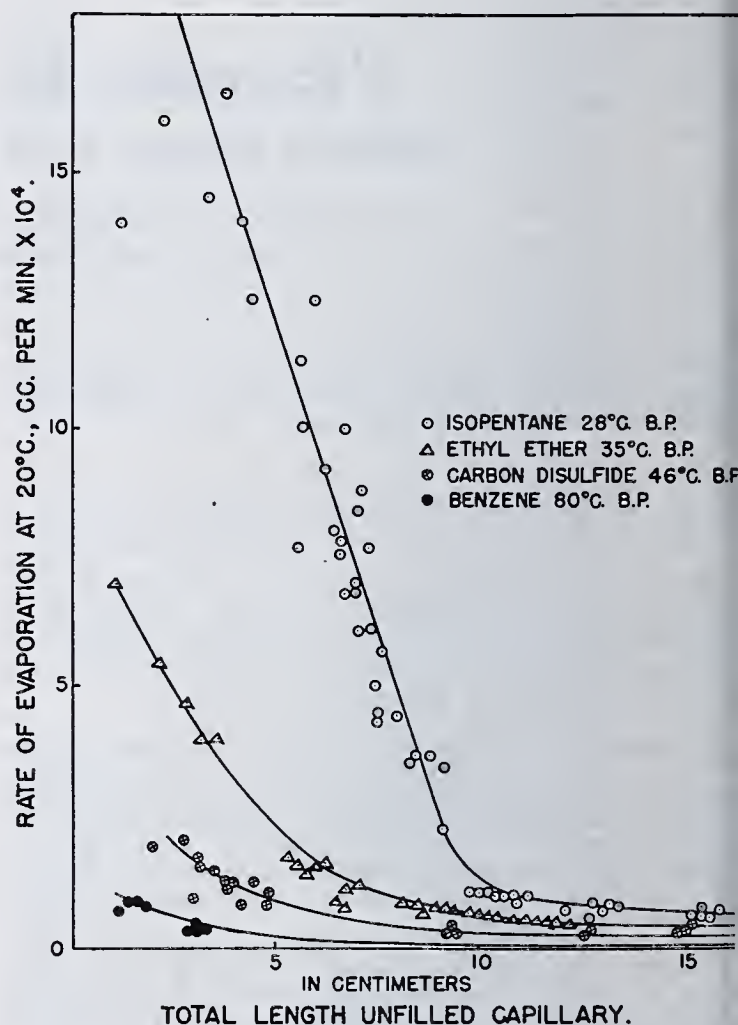


Figure 2. Evaporation Rates

The pycnometer is filled in an upright position by placing the hooked tip in a vial containing the liquid until the liquid level reaches scale mark 4 on the bulb side. Liquid level must be on the scale when the pycnometer contents are at  $20^{\circ}\text{C}$ . ( $68^{\circ}\text{F}$ ). In hot weather ( $100^{\circ}\text{F}$ .), the contraction to  $68^{\circ}\text{F}$ . on a 3-ml. pycnometer of small bore is approximately 10 cm. If room temperature is below  $68^{\circ}\text{F}$ . or the sample is much below the temperature, due allowance should be made in the filling level. The level of liquid in the vial must be very near the top. Petroleum will fill a 3-ml. pycnometer in less than a minute. More viscous samples will fill more slowly. This pycnometer is not recommended for samples more viscous than gas oil of 50 second Saybolt viscosity at  $100^{\circ}\text{F}$ . (about 7.4 centistokes).

The pycnometer is removed from the sample bottle, and the hook which had been immersed in the sample is cleaned with cloth moistened with benzene and wiped dry. The weight is then obtained within  $\pm 0.0001$  gram.

The pycnometer is placed for 10 to 15 minutes in a vertical position in a glass jar thermostat held at  $20.00^{\circ} \pm 0.05^{\circ}\text{C}$ . and allowed to reach that temperature. A variation of  $0.05^{\circ}\text{C}$ . equivalent to approximately 0.00004 gram ml. per ml. for gas



in hydrocarbon. A more general expression for the temperature coefficient of density of hydrocarbons may be obtained from the correlation of Lipkin and Kurtz (18).

The meniscus levels are read on both arms of the instrument within one half of the smallest scale unit without removing the pycnometer from the bath. To avoid undue volatilization losses, the elapsed time between weighing and reading the meniscus levels should not be over 15 minutes. The volume in milliliters corresponding to the sum of the readings is read from the calibration curve:

$$\text{Density in air} = \frac{\text{weight of sample}}{\text{observed volume}} \quad (2)$$

Equation 1 gives the corrections to be added to the determined density to take care of air buoyancy and to obtain density in vacuum.

**4. HIGHLY VOLATILE MIXTURES.** This procedure is to be used on highly volatile mixtures because it prevents or reduces change in the compositions of the sample by the selective volatilization of the lower boiling components while the pycnometer is being filled and while it attains balance temperature.

The sample is cooled to ice temperature, charged to the pycnometer, and the pycnometer and contents are allowed to come to temperature, 20° C. The volume is read, the instrument removed from the bath, the outside cleaned and dried, and the pycnometer plus sample weighed. The sample is then flushed out in the pycnometer with benzene, and the pycnometer is dried, weighed empty.

Either procedure may be used on pure compounds of high volatility, since selective loss of one component is not involved. Procedure A is more convenient for samples which are not extremely volatile.

In both procedures there is a time interval between the measurement of weight and the measurement of volume. If volatilization occurs between these measurements in the case of procedure A a high density will result, since weight is measured first. In the case of procedure B, since volume is measured first, low densities will result from such vaporization. Such losses are negligible except in the measurement of very volatile materials such as isopentane in pycnometers of small volume such as 0.3 ml. If vaporization losses are suspected, the density should be determined by both procedures and averaged.

Table I. Deviations in Density Observed Using Pycnometers of Different Sizes<sup>a</sup>

Operator	Material Examined	d 20/4	0.32-Ml. Pycnometer	1.2-Ml. Pycnometer	3-Ml. Pycnometer	5-Ml. Pycnometer
1	Isopentane	0.6197	+0.0019	+0.0014	+0.0006	-0.0001
1			+0.0011	+0.0010	±0.0000	-0.0001
2			+0.0084	+0.0025	+0.0005	-0.0002
2			+0.0040	+0.0011	+0.0001	-0.0001
	Av.		+0.0038	+0.0015	+0.0003	-0.0001
1	Ethyl ether	0.7140	+0.0024	+0.0002	-0.0002	-0.0005
1			+0.0022	+0.0006	±0.0000	-0.0001
2			+0.0044	+0.0013	+0.0004	±0.0000
2			+0.0006	+0.0001	-0.0001	+0.0000
	Av.		+0.0024	+0.0005	-0.0002	-0.0001
1	300-400° F. naphtha	0.7975	-0.0003	-0.0005	-0.0001	+0.0001
1			.....	-0.0003	-0.0002	-0.0001
2			+0.0004	-0.0007	-0.0003	-0.0003
2			-0.0004	-0.0006	±0.0000	-0.0002
	Av.		-0.0004	-0.0005	-0.0001	-0.0002
1	(50% isopentane) 50% 300-400° F. naphtha	0.7128	-0.0015	+0.0005	-0.0002	±0.0000
1			-0.0022	+0.0003	+0.0002	-0.0001
2			-0.0069	+0.0009	+0.0002	+0.0004
2			+0.0019	+0.0012	+0.0003	±0.0000
	Av.		-0.0031	+0.0007	+0.0002	+0.0001
1	Gas oil 50 sec. Saybolt at 100° F.	0.8304	-0.0032	-0.0005	±0.0000	+0.0001
1			-0.0028	-0.0008	-0.0002	+0.0001
2			-0.0014	-0.0005	-0.0001	±0.0000
2			+0.0001	-0.0010	-0.0003	-0.0003
	Av.		-0.0019	-0.0007	-0.0002	-0.0001

<sup>a</sup> Obtained using procedure A and early design of pycnometer with both arms straight. Similar data obtained with bent-arm pycnometer check these data. Earlier tests are given, since they are more complete than recent tests.

## PRECISION

Tests of the precision of the pycnometer using procedure A are shown in Table I. (Results obtained with procedure B are of comparable accuracy.) Two operators determined the densities of isopentane, ethyl ether, a naphtha, a mixture of isopentane and a naphtha, and a gas oil. Each operator made duplicate determinations. The data showed that, with the 3- or 5-ml. pycnometer, precision does not depend on the operator, and that the 5-ml. size is precise to ±0.0001, the 3-ml. to ±0.0002, the 1-ml. to ±0.001, and the 0.3-ml. to ±0.002 gram per ml. over the entire viscosity and volatility range between pentane and light gas oil.

The sources of the larger errors in determining densities with the smaller pycnometers are shown in Table II. The effect of the various possible errors on the final determination of the density is given. With the 1-ml. and the 0.3-ml. pycnometers, errors in the scale readings and weighings are important. Errors due to temperature control and air buoyancy are the same with all size pycnometers, as indicated in the table. The 3- and 5-ml. pycnometers are recommended for routine use.

Table II. Errors in Determined Densities of Ethyl Ether Due to Errors of Observation

Volume of Pycnometers <sup>a</sup> ML.	1° C. Error in Temperature G./ml.	0.5 Mg. Error in Weighing G./ml.	0.5 mm. Error in Scale Reading G./ml.
0.25	0.0011	0.0020	0.0011
1	0.0011	0.0005	0.0003
3	0.0011	0.0002	0.0001
5	0.0011	0.0001	0.0001

<sup>a</sup> Capillary diameter 1.0 mm.

## ACCURACY

The data in column 3, Table I, on the density of the materials were obtained by a skilled operator with the 5-ml. pycnometer, and are believed to be accurate within ±0.0001 gram per ml. If this is true, the data in Table I constitute a test of accuracy as well as of precision. As a further test of absolute accuracy of the authors' method, the density of a carefully purified sample of benzene was determined on a pycnometer calibrated with water. The density at 20° C. was found to be 0.87895. This result checks the literature value of Timmermans (28) of 0.87895 corrected from 15° C. and the value of 0.87891 determined by Wojciechowski (32) corrected from 25°, using the temperature coefficient of density values determined by Timmermans.

## SPEED

Each determination requires 10 to 15 minutes of the operator's time, and an elapsed time of 20 to 30 minutes. With a damped balance, 10 minutes of operator's time per sample is sufficient.

## ACKNOWLEDGMENT

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# Apparatus for Measuring the Gas Permeability of Film Materials of Low Permeability

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Apparatus is described for measuring the gas permeability of film materials having permeabilities as low as 0.001 cc. (at standard temperature and pressure) per 100 square inches per 24 hours. The low range for previously reported methods (water vapor permeability and balloon fabric permeability) is about 100 cc. per 100 square inches per 24 hours. The apparatus combines simplicity of design, simplicity of manipulation, and high sensitivity in a unit which can be fabricated in most machine shops. The measurements are made under conditions of one atmosphere pressure differential.

THE packaging of some foods, drugs, chemicals, etc., requires flexible films and paper coatings of very low permeability to fixed gases, particularly oxygen. The need for film having a permeability of not more than 0.25 cc. per 645 sq. cm. (100 square inches) per 24 hours has been pointed out by Elder (3). This figure is entirely out of the range usually measured for balloon fabrics (5), where permeabilities range from 100 cc. per 100 square inches per hour and up. The customary measurements of water vapor permeability (1, 2, 4) of packaging films are also in a relatively high range—for example, a "good" water vapor barrier will have a permeability figure of 0.1 gram or 125 cc. per 100 square inches per 24 hours. The apparatus described in this paper is designed for measurements in the range 0.001 to 1000 cc. per 100 square inches per 24 hours, and primarily for the measurement of the fixed gases, although with some modification it might be also used for water vapor permeabilities. The purpose of this publication is to make available to others the means which the author has developed for testing packaging films of very low permeability.

The mechanism of gas transmission through solids has not been explained thoroughly. Oswin discusses this briefly (4). If the gas passed through small pores or openings in the solid, the situation would be a relatively simple one; the permeability of a film to various gases could be predicted from the known laws of gas flow through orifices. However, such is not the case. It is known that polyvinyl alcohol film is permeable to water vapor but not to oxygen, and Pliofilm is permeable to carbon dioxide but

relatively impermeable to oxygen. Perhaps a process of solution of gas in the film on one side, diffusion through the film, and evaporation from the film on the other side takes place in some cases.

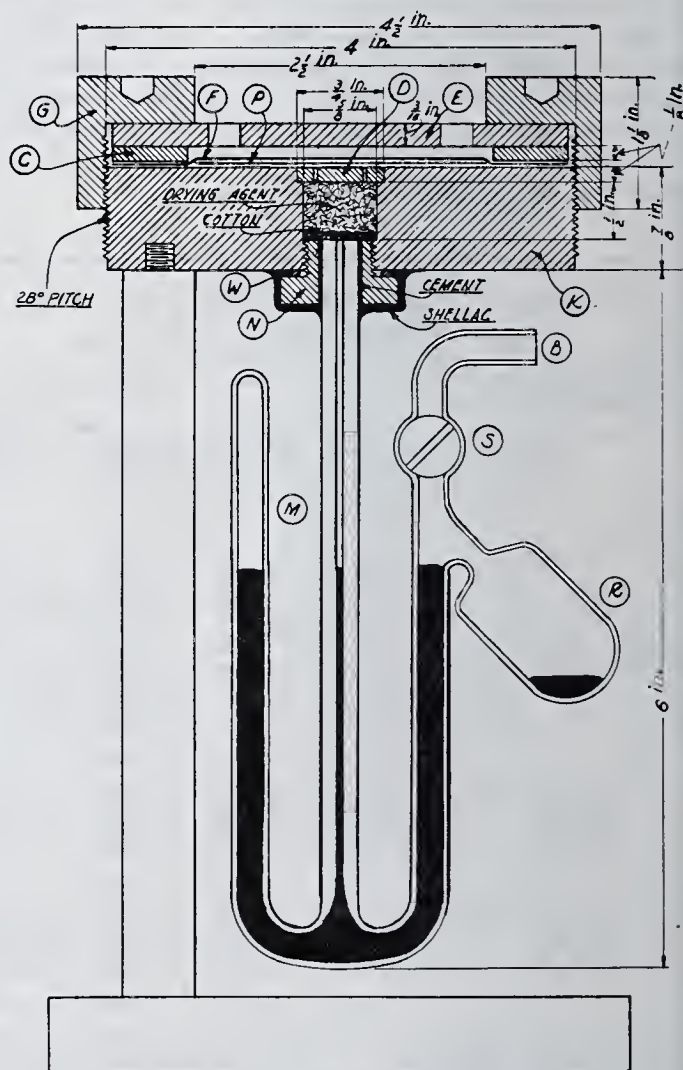


Figure 1. Diagram of Apparatus



The mechanism of gas transmission through film determines to some extent the conditions under which a film should be tested. Two factors are involved: the difference in total pressure on the two sides of the film and the difference in partial pressure of the gas being tested on the two sides of the film. If permeability is due to gas flow through orifices in the film, the difference in total pressure is of primary importance and the difference in partial pressure is of secondary importance. If the permeability is due to solubility of the gas in the film, the difference in total pressure is of no importance, but the difference in partial pressures is important. In the apparatus described in this article, the test is made under the conditions of atmospheric pressure of the gas being tested on one side of the film and zero pressure of all gases on the other side.

The apparatus, shown in Figures 1 and 2, combines simplicity of design, simplicity of manipulation, and high sensitivity in a unit which can be fabricated in most machine shops. Essentially the method involves measuring changes in pressure inside a small evacuated space due to gas passing into that space through the film sample being tested.

CONSTRUCTION OF APPARATUS

The manometer, *M*, is held in place by the nut, *N*, to which it is sealed with Cenco Plicene cement or other sealing compounds of good adhesive properties and low vapor pressure. The hole through the nut is bored nearly the same diameter as the glass tube, thereby minimizing the amount of cement necessary for this seal. The nut is screwed up against the thin rubber washer, *W*, and the entire joint is coated with shellac to ensure a vacuum-tight seal.

Manometer *M* serves the dual purpose of recording the pressure change and of evacuating the space inside the apparatus. For evacuation, the entire apparatus is tipped so that the mercury runs over into the reservoir, *R*; then the apparatus is evacuated through tube *B* with stopcock *S* in the open position. After evacuation, *S* is closed and the apparatus is tipped again so that the mercury runs from *R* into *M*. In case air leaks through *S* during a test, the mercury in that arm of the manometer will be depressed, thus serving as an indicator of leakage at this point without spoiling the test. The center arm of *M* is made of capillary tubing of about 1.5-mm. internal diameter for the purpose of reducing the volume of gas space inside the apparatus and thereby increasing its sensitivity. The rest of the manometer may be made of tubing of any convenient size. The scale used in measuring the height of the mercury is not shown. A piece of millimeter cross-section paper held behind the manometer serves very well for the purpose.

The upper part of the hole in the center of the disk, *K*, is covered with a small metal disk, *D*, which has four small holes about 1 mm. in diameter for passing gas into the manometer system. The top surface of the small disk is flush with the top surface of the large disk, *K*, presenting a continuous smooth surface for mounting the film, *F*.

The drying agent absorbs all moisture which passes through *F*, thus eliminating this gas as a source of error in gas transmission measurement. Dehydrite (anhydrous magnesium perchlorate) diluted with a small quantity of Indicating Drierite (anhydrous calcium sulfate) serves admirably. A screen fraction passing 14-mesh and retained on 40-mesh has been found to be a convenient size. In general, any drying agent which will reduce the water vapor pressure inside the apparatus to less than 0.5 mm. of mercury will serve. A small plug of cotton over the top of the manometer tube will prevent particles of the drying agent from falling into the manometer.

MOUNTING SAMPLES

The film sample, *F*, is mounted on the apparatus as shown in the diagram. A piece of filter paper, *P*, is placed between the film and *K* to provide a porous medium for gas leaking through the film to travel to the openings in *D* and pass through the drying agent into *M*. The part of *F* overlapping *P* and in direct contact with *K* is sealed to the disk by means of a thin film of heavy stopcock grease having a low vapor pressure. This area of the film is held in place by the rubber gasket, *C*.

Disk *E* has two small holes diametrically opposite one another and near the inside circumference of the metal ring, *G*. When measuring air transmission, these openings are left open so that the space between *E* and *F* is filled with air. When it is desired to measure the gas transmission for any other gas, a small rubber

stopper with a glass tube is inserted into each of these holes. The space between *E* and *F* is then flushed out with gas to be measured by passing the gas in one opening and out the other. It has been shown that the moisture content of fixed gases markedly affects their rate of transmission through some film materials (4). This factor may be controlled by conditioning the gas to be tested before it is passed through the apparatus.



Courtesy "Modern Packaging"

Figure 2. Apparatus

In mounting multi-ply film having one or more plies of a porous material—for example, a film laminated between two pieces of paper—it is practically impossible to obtain a vacuum-tight seal using stopcock grease between the paper surface of the sample and *K*. In mounting such films, use has been made of a plasticized tar as shown in Figure 3. An edge coating extending about 0.6 cm. (0.25 inch) from the circumference is applied to both sides of the disk of film to be tested by dipping in the plasticized tar (Figure 3). The tar-coated disk of material is mounted directly on *K* without the use of stopcock grease. When cap *G* is screwed into place, the plasticized tar is spread out by the compressive forces and makes a vacuum seal between *F* and *K*. Carbowax 1500 is a suitable plasticizer for the tar when used at the level of about 5 per cent.

CALCULATIONS

Gas transmission measurements are conveniently recorded as cubic centimeters of gas at standard conditions of temperature and pressure transmitted per 100 square inches of surface per 24 hours. Such a figure is given by the following expression:

$$\frac{P}{760} \times V \times \frac{273}{T} \times \frac{100}{A} \times \frac{24}{\text{hours}}$$

where *P* = the absolute pressure inside the apparatus as measured on the manometer in millimeters

*V* = total volume of the inside of the apparatus in cubic centimeters

*T* = temperature in degrees absolute

*A* = surface area of test sample (area of filter paper *P* in square inches)

hours = time of test

The value of *V* may be calculated with sufficient accuracy (within 5 per cent) from the known dimensions of the apparatus, making a correction for the volume of the drying agent used.



It is equal to the sum of the volume of the holes in  $D$ , the volume of the circular opening containing the drying agent minus the volume of the drying agent, and the volume of the capillary bore of the center stem of the manometer down to the mercury level in this stem. The volume of the pore space in  $P$  amounts to about 0.05 cc. and the volume change due to a mercury level change of 20 mm. in the center stem of the manometer amounts to about 0.04 cc. These errors in volume are neglected.

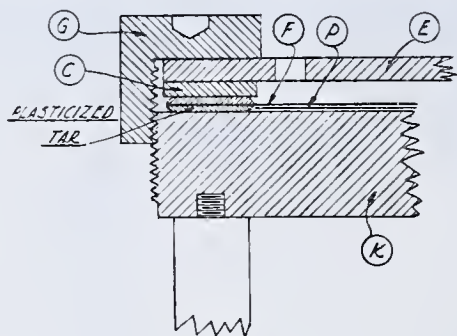


Figure 3

For the apparatus diagrammed in Figure 1, the value of  $V$  is about 2 cc., and the value of  $A$  is 23 sq. cm. (5 square inches). At a temperature of  $30^\circ\text{C}$ .,  $T = 303^\circ$  absolute. Substituting these values in the above equation, we get the following working equation:

$P/\text{hours} \times 1.14 = \text{cc. of gas at standard conditions of temperature and pressure transmitted/100 square inches/24 hours}$

#### SENSITIVITY, ACCURACY, AND PRECISION

The actual time required for a test with this apparatus varies with the permeability of the film being tested and the limits within which it is desired to determine the gas transmission rate.

For example, it might be desired to know whether a film sample has a transmission rate greater or less than 0.25 cc. per 100 square inches per 24 hours. Using the above equation and constants for the apparatus herein described, this transmission rate will produce a pressure change in the manometer of 0.5 mm. in about 2.5 hours: 0.5-mm. change is easily read on the manometer; therefore, the failure of the manometer to change 0.5 mm. in 2.5 hours' time is sufficient evidence that the gas transmission rate is less than 0.25 cc. per 100 square inches per 24 hours. Should the time of the test be extended five times as long, to 12.5 hours, the failure of the manometer to change 0.5 mm. is sufficient evidence that the gas transmission rate is less than 0.05 (0.25/5) cc. per 100 square inches per 24 hours. Further extending the time of the test thus further reduces the figure for the maximum transmission rate of the sample.

With films of high permeability, the mercury in the manometer will drop several millimeters in an hour's time. With such films, therefore, the test need not be extended more than a few hours.

Occasionally film samples contain material, such as plasticizers, which have appreciable vapor pressures. Such materials will vaporize into the apparatus and depress the mercury in the manometer. This may be mistaken for gas transmission through the film, but may be distinguished from gas transmission by the fact that the pressure will reach a constant value, the rate of change of pressure decreasing as this value is approached. It is therefore necessary to make readings of the manometer at intervals during the test and calculate the rate of manometer change for each interval. If the rate of change is constant, it is due to gas transmission through the film, but if it is a decreasing rate of change, the change is due at least partly to vaporization of some volatile material from the film.

The required accuracy is not very great for measurements of permeability of film packaging materials where values might vary a millionfold among various materials and a thousandfold among various samples of the same material. It is estimated that meas-

urements with this apparatus are accurate to within 15 per cent. The temperature, time, and manometer reading may be determined within a few per cent. The principal factors affecting the accuracy are the volume of the apparatus and the area of the test sample. It is estimated that these are easily determined in the manner described within 10 per cent accuracy. The precision or reproducibility of measurements on duplicate samples of the same material is generally about 10 per cent.

#### EXAMPLES

The following examples will serve to typify the data which may be obtained with this apparatus:

1. Air transmission for an impermeable sample (a sample of laminated glassine at 60 to 80 per cent relative humidity).

In a period of 36 hours, no change was observed in the manometer. Assuming that a minimum change of 0.5 mm. can be detected on the manometer, the rate of change of the manometer was not greater than  $0.5/36$  or 0.0139 mm. per hour. The gas transmission was, therefore, not greater than  $0.0139 \times 1.14$ , or 0.016 cc. per 100 square inches per 24 hours.

2. Air transmission for an impermeable sample containing a volatile plasticizer (a polyvinyl alcohol coating at low humidity).

The manometer changes during the indicated time interval were as follows:

Time Interval Hours	Manometer Change Mm.	Rate of Change Mm./hour
0-1	2	2.0
1-2	1.5	1.5
2-4	2	1.0
4-10	3.5	0.58
10-40	0.5	0.017
Total	9.5	

It is evident from the above data that a material having a vapor pressure of about 9.5 mm. is evaporating from the sample and that equilibrium is nearly established after 10 hours. The rate of change of the manometer during the last 30 hours of the test was 0.017 mm. per hour. Therefore, the gas transmission rate for the sample was not more than  $0.017 \times 1.14$  or 0.019 cc. per 100 square inches per 24 hours.

3. Air transmission for a permeable sample (a thin coating of polyvinyl alcohol).

The manometer changes during the indicated time interval were as follows:

Time Interval Hours	Manometer Change Mm.	Rate of Change Mm./hour
0-1	1.5	1.5
1-2	1.5	1.5
2-4	3.0	1.5
4-10	9.5	1.58
10-18	11.5	1.45
Total	18	Av. 1.5

The constancy of the rate of change figures indicates that this sample is permeable to air. The rate of gas transmission is  $1.5 \times 1.14$ , or 1.71 cc. per 100 square inches per 24 hours.

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Because of critical shortages, the AMERICAN CHEMICAL SOCIETY has been forced to cut its use of paper to an absolute minimum. It will no longer be possible to print the customary number of extra copies to supply demands for volumes and sets in subsequent years. Therefore, it is suggested that subscribers who do not bind their journals save current issues for later sale.



# Design of Large-Size Laboratory Extraction Glass Apparatus

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A SIMPLE large-capacity extraction apparatus has been described by Smallwood (2).

The frequent need for extraction apparatus of larger capacity than the conventional Soxhlet extractors for research as well as for small pilot production justifies a description of the improved all-glass apparatus which has been in satisfactory operation in this laboratory for a number of months.



Figure 1

The apparatus is shown in Figure 1, after removal of the insulation. It is designed for operation at either ordinary or reduced pressures and is equipped to permit the collection of all engineering data required for pilot work. It is made of standard Pyrex parts with Ace spherical joints, size 35/25. The boiler is a three-neck 12-liter flask installed in a Glas-Col heater provided with proper input control (rheostat and ammeter) and pyrometer. The extractor has a total capacity of 8 liters. In order to obtain a sufficient speed of extraction the hot solvent vapor is fed at the top of the 600-mm. Allihn condenser, instead of the bottom, as in the conventional Soxhlet. Experiment has shown that flooding occurs with the conventional design at a distillation rate of 3 liters per hour at ordinary pressure and 1.5 liters per hour at 74.1 mm. hg. (27-inch) vacuum in the presence of alcohol vapor, with a condensing area of 640 sq. cm. cooled with brine, whereas the present design permits an extraction of 5.5 liters per hour at ordinary pressure and over 4 liters per hour in vacuum with a condensing area of only 350 sq. cm. When used under vacuum, excessive evaporation of the solvent in the extractor is prevented by a coack-condenser inserted in the vacuum line (350-sq. cm. Allihn Pyrex vertical condenser) at the level of the side connection of the 8-liter bell jar forming the extractor.

Inasmuch as the sudden emptying of the large extractor into the hot boiler could be dangerous, particularly with very volatile solvents, the return U-tube is provided with a glass stopcock which permits either controlled periodical or continuous circulation of the solvent. A sampling outlet is provided at the bottom of the extractor.

The condensers can be cooled with either water or brine, and the piping circuit includes the necessary valves and measuring devices for determination of the heat balance.

Table I indicates the performance of the apparatus in two different experiments.

The volatility involved in calculating the over-all theoretical plate number has been calculated from the recent data of Beebe and co-workers (1): For  $x_f = 24.10$  and  $x_0 = 56.75$  the overhead

temperature was always lower than 39.3° C., the difference being probably accounted for by the presence of noncondensable gas in the vapor phase, which is difficult to avoid in apparatus of relatively large size. Despite the length of the vapor line, the apparatus appears well suited for use with mixed or diluted solvents.

Some determinations of the approximate heat balance have been made and one typical result is reported in Table II.

Table I. Performance of Apparatus

Head Temperature ° C.	Boiler Temperature ° C.	Extract Temperature ° C.	Absolute Pressure Mm. Hg	Over-head Mole %	Bottom Mole %	Plate No. <sup>a</sup>
32	36	27	109	73.0	23.5	1.4
29	35	26	100	62.8	23.5	1.1

<sup>a</sup> Over-all theoretical plate number. Although a certain reflux takes place from the top of the vapor line downward, no liquid is ever carried back to the still pot when the heat input is correctly adjusted. Therefore the phases in equilibrium considered for the calculation of the "over-all theoretical plate number" were the still pot liquid composition and the distillate composition, respectively. No flow measurement is involved in this calculation. However, the rate of distillation can be determined by measuring the time required to fill the extractor whose capacity is known; this operation requires shutting off the stopcock on the return U-tube for only a short time.

The data reported show the excellent performance of the apparatus. They demonstrate the possibility of obtaining a rate of extraction much faster than hitherto reported with large all-glass laboratory apparatus, while at the same time maintaining a satisfactory and economical heat balance so that the extraction can be carried out under conditions more closely resembling those of plant operation.

Table II. Heat Balance

Heat Input from Distillate		Heat Output (to Brine)	
A. Heat of condensation		Rate of brine flow, liters per min.,	
Rate of distillation, ml. per min., 80		5.6	
Distillate, sp. gr., 0.840		Brine, sp. gr., 1.20	
Ethanol, mole %, 62.8		Brine, sp. heat, 0.7	
Water, mole %, 37.3		Input temperature, ° C., 8.0	
Sp. heat of ethanol, 204.26		Temperature increase, ° C., 8.1	
Sp. heat of water, 579.3		Weight, gal. of water, lb., 8.33	
A = 23,103.96 calories per min.		Heat output, 81.4 B.t.u. or 20,462.4 calories per min.	
B. Heat of cooling of distillate			
Temperature decrease, ° C., 29-26° = 3°			
Sp. heat of 62.8 mole % of ethanol at 23° C., calories per gram, 72.0			
B = 145.15 calories per min.			
Total heat input, 23,249.11 calories per min.		Balance <sup>a</sup> , 2787 calories per min. or 11.9%	

<sup>a</sup> "Balance" represents summed effect of heat losses from the still pot and the vapor line, the thermometers, various experimental uncertainties, and some simplifying assumptions made in calculation of the quantities involved.

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PRESENTED before the Division of Industrial and Engineering Chemistry at the 105th Meeting of the AMERICAN CHEMICAL SOCIETY, Detroit, Mich.



# A Continuous Liquid-Liquid Extractor

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RECENTLY Kieselbach (1) introduced a new principle into the field of liquid-liquid extractors for use with mixtures which tend to emulsify. His extractor, using air agitation and a settling chamber for the separation of any emulsion formed, made possible the rapid extraction of solutions formerly taking as long as several weeks. However, Kieselbach's extractor has a number of disadvantages, chief among which are the facts that a separate extractor is necessary for each desired volume of solution to be extracted and that very stable emulsions do not separate in the narrow settling chamber.

The apparatus herein described was an attempt to increase the utility of Kieselbach's extractor for use with strongly emulsifying sulfite waste liquor reaction mixtures varying in volume from 250 cc. to several gallons. The extractor unit is actually an adapter to be used with standard-taper glass bottles or flasks. It is made from readily available stock glassware and requires a minimum of glass blowing.

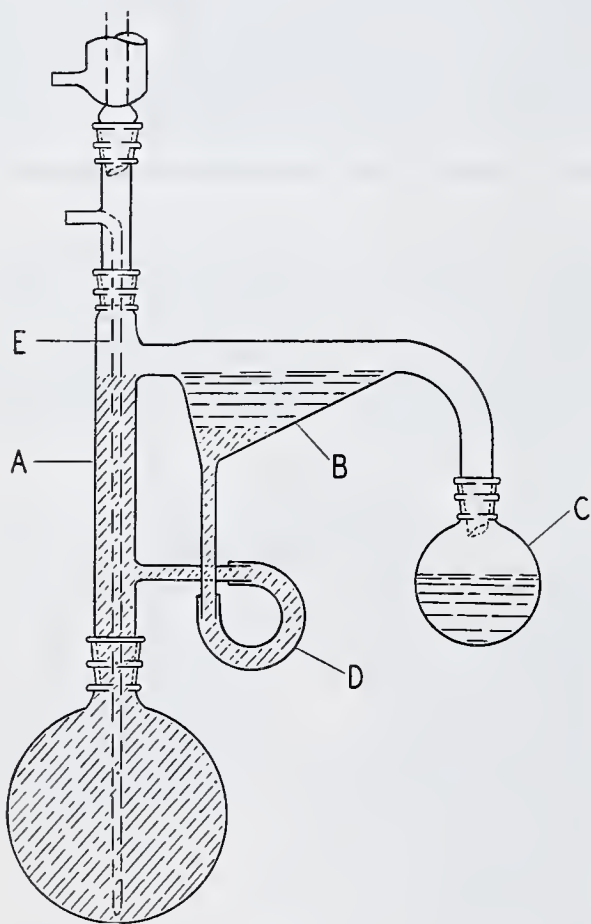


Figure 1

The operation of the complete extractor assembly drawn to scale in Figure 1 is identical to Kieselbach's. The extractor unit was used successfully for extracting solutions contained in vessels ranging from 250-cc. flasks to 12-liter bottles.

The mixing chamber, A, was made by sealing together two standard-taper glass joints. The upper joint in this case was 24/40, although any size compatible with other equipment is satisfactory. The lower joint was 29/42; because of the possibility of constriction, a smaller standard-taper joint should not be used. Both standard joints were connected to 28-mm. outside diameter tubing. A 500-cc. Erlenmeyer flask served as the settling chamber, B, and was connected to the mixing chamber by a short piece (30 mm.) of 25-mm. outside diameter tubing. A 24/40

standard-taper joint was used for the connection to the boiling flask, C. Tubing of at least 10-mm. outside diameter should be used for the glass part of the trap, D; otherwise, the passage of very slowly breaking emulsions is impeded. The trap itself was made of a short length of fairly thick-walled neoprene tubing. The inner tube, E, was made by sealing a piece of 8-mm. outside diameter glass tubing to a stock gas inlet adapter. The length of the tube depended upon the size of the flask containing the material to be extracted. If a number of gas inlet adapters are not available, a 10/30 standard joint may be sealed to the stock adapter and a number of 8-mm. tubes with standard 10/30 joint of lengths to fit various flasks or bottles may be used.

The completion of the extraction is usually determined by the change in color taking place in the settling chamber. However, in the case of extractions of colorless substances, samples of the solvent layer in the settling basin may be taken periodically by lowering the solvent solution interface (by withdrawing a little solution through the gas inlet tube, E) and the appropriate use of several screw clamps on the rubber trap. (Richard Kieselbach, after reviewing the paper, suggested that the settling chamber might be provided with a tubulature, close to the point which used to be the neck of the Erlenmeyer flask, which would permit sampling without interruption of the operation of the extractor. This should prove a valuable modification of the present apparatus when dealing with colorless solutions.)

A glass T in the rubber trap may be used if a number of solutions are to be encountered. When the extraction is complete, the solvent in the settling basin may be drawn off in the manner described above. These advantages make the rubber trap preferable to the all-glass U-trap. In addition, the glass blowing is greatly simplified. An Erlenmeyer flask was used for the settling chamber because many fairly stable emulsions do not break in the small-diameter settling basin of the earlier apparatus. The shape of the Erlenmeyer flask is admirably suited for this purpose because the slopes of its base and sides (when in the position shown in Figure 1) facilitate rapid separation of the two liquid phases. Furthermore, this design is relatively compact. For maximum applicability, the seals at both ends of the Erlenmeyer flask should be at the same level. The size of the flask depends upon the nature of the solutions encountered. For fast-breaking emulsions a 250-cc. flask may be used, thereby holding up less solvent in the settling chamber. If a large mixing chamber is used with a correspondingly increased air stream, a larger settling chamber should be used. The specifications of the entire apparatus are flexible.

When extracting materials subject to oxidation by air a stream of inert gas should be used for agitation. Furthermore, when extracting gas-saturated solutions (such as bicarbonate or bisulfite solutions), carbon dioxide or sulfur dioxide, respectively, may be used advantageously as the agitating gas. In extractions of large volumes of solutions with corresponding increases in time, the loss of solvent due to extrainment in the exit gases may become appreciable, and more solvent may have to be added to the boiling flask. In this case advantage may be taken of a two-necked boiling flask. A long efficient reflux condenser should always be used. An active carbon trap is useful for recovering large amounts of solvents.

A large apparatus, using semiball joints, for use with 22-, 50-, and 72-liter flasks was fabricated according to specifications of the Scientific Glass Apparatus Co., Bloomfield, N. J.

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# Determination of Small Amounts of Acrylonitrile in Air

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THE recent widespread use of acrylonitrile in the manufacture of one type of synthetic rubber has made it imperative that a quantitative method for its determination in air be developed. The toxicity experiments conducted by the U. S. Public Health Service (1) indicate that the maximum allowable limit for acrylonitrile is in the neighborhood of 20 p.p.m. Consequently, an analytical method must be accurate to at least that concentration. As far as is known no analytical method has yet been reported. The Raleigh-Jeans gas interferometer has been used for concentrations above 90 p.p.m. Below this range the results are questionable (2). In addition, the gas interferometer is not very satisfactory for field use where mixtures of vapors are likely to be encountered.

## METHOD

The method developed in this laboratory depends upon a modified Kjeldahl reaction in which the absorbing solution containing the acrylonitrile is made strongly alkaline with sodium hydroxide and then oxidized with hydrogen peroxide. Upon refluxing, the acrylonitrile is converted quantitatively to ammonia, which is distilled over and collected in a standard acid solution. The amount of ammonia evolved is determined by titration of the excess acid. The acrylonitrile vapors are absorbed in cold concentrated sulfuric acid contained in a suitable absorption trap. This method is limited by the fact that there can be present in the contaminated air no hydrogen-bearing compounds other than acrylonitrile.

## PROCEDURE

**SAMPLING.** Two absorption traps (Figure 1) are filled to a depth of about 2.5 cm. (1 inch) with glass beads, and 5 ml. of concentrated sulfuric acid are added to each trap. The traps are connected in series and put in a water-ice bath. The air sample is drawn through at maximum rate of 0.4 liter per minute. The rate of sampling is accurately measured by means of a rotameter. The volume of air sampled should be such that the total sample consists of approximately 6 mg. of acrylonitrile. The sample is then washed into the reflux flask (Figure 2), and 1 gram of copper acetate is added as an inhibitor to prevent polymerization.

**ANALYSIS.** Twenty-five milliliters of 0.025*N* sulfuric acid are added into the titration beaker and diluted with distilled water until the bubbler is at least 1.25 cm. (0.5 inch) below the surface. The reflux flask containing the sample is put into place and the system is completely closed. The acid sample is made alkaline by adding 50 ml. of 50 per cent sodium hydroxide to the closed system by means of the separatory funnel. The residual sodium hydroxide in the reflux condenser is washed down with 10 ml. distilled water, and 20 ml. of 30 per cent hydrogen peroxide is then added slowly from the separatory funnel. When the reaction is completed, the sample is refluxed gently for 30 minutes. At the end of the reflux time, the water is drained from the reflux condenser and approximately one half of the sample is distilled over. The second condenser is then washed down with distilled

Table I. Accuracy of Method over Wide Range of Concentrations

Theoretical Concentration P.p.m.	Sampling Rate L./min.	Sampling Time Min.	Yield		
			Mg.	P.p.m.	%
400	0.4	20	6.54	394	98.5
200	0.4	35	5.51	189	94.6
100	0.4	75	6.12	97.9	97.9
50	0.4	150	6.29	50.6	101.2
25	0.4	180	4.50	30.0	120.0

water, and the excess sulfuric acid is titrated with 0.01*N* sodium hydroxide, using methyl red as the indicator.

The concentration of acrylonitrile is calculated as follows:

$$\text{P.p.m.} = (25.00N_A - N_B V_B) \times \frac{1}{V_S} \times C$$

when  $N_A$  = normality of  $\text{H}_2\text{SO}_4$

$V_B$  = ml. of NaOH used in titration

$V_S$  = volume of air sampled, liters

$N_B$  = normality of NaOH used in titration

$C$  = 22,400 corrected to sampling pressure and temperature

## DISCUSSION

The analysis is based upon Radziszewski's reaction (3). The mechanism is as follows:

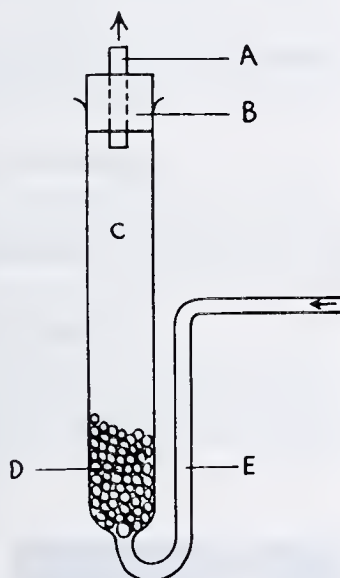
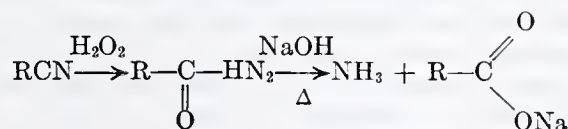


Figure 1. Absorption Trap

- A. Glass outlet tube
- B. One-hole rubber stopper
- C. 0.25-inch test tube
- D. Glass beads
- E. Glass inlet tube

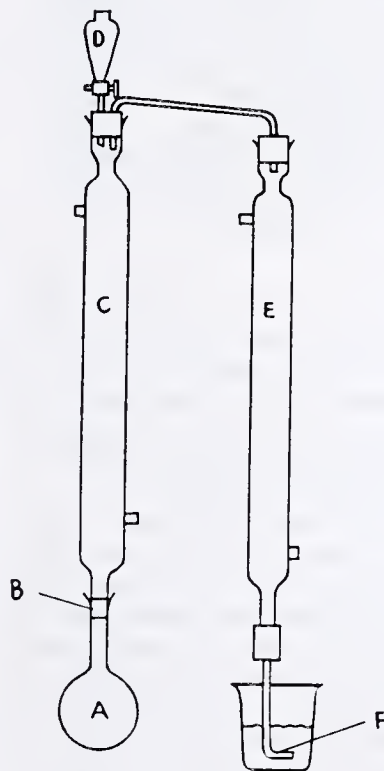


Figure 2. Apparatus for Determination of Acrylonitrile

- A. 200-cc. balloon flask
- B. Ground-glass joint
- C. Reflux condenser
- D. Separatory funnel
- E. No. 2 condenser
- F. Sintered-glass bubbler

The use of hydrogen peroxide reduces the reflux time from 4 hours to 0.5 hour, and also drives the reaction to completion. Low yields due to polymerization of the acrylonitrile are prevented by the addition of copper acetate as an inhibitor, and by limitation of the size of the sample. A sample containing approximately 6 mg. of acrylonitrile proved to be most satisfactory because it is dilute enough to prevent polymerization and yet large enough to give an accurate analysis. The maximum sampling rate of 0.4 liter per minute is very critical for the type of absorption equipment described in this article. A higher rate will result in loss of sample.



Table I contains the results of the analyses of known concentrations of acrylonitrile vapors, prepared in a gas chamber of 1000 liters' capacity by the introduction of measured amounts of liquid acrylonitrile. The various concentrations were chosen to indicate the accuracy of the method over a wide range.

Over the entire range of concentrations the only variable is the time of sampling. The large relative error in the analysis of the 25 p.p.m. samples is misleading, since the actual error is only 5 p.p.m. The principal reason for the error in the results of the low concentrations is the difficulty of measuring the exceedingly small amounts of liquid acrylonitrile required in making up these concentrations in the 1000-liter gas chamber available.

# Spectrophotometric Determination of Iodine Liberated in the Oxidation of Carbon Monoxide by Iodine Pentoxide

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A spectrophotometric method for measuring the iodine liberated in the oxidation of carbon monoxide by iodine pentoxide is described. The iodine concentration was measured at 350 millimicrons against a water blank, using a Coleman photoelectric spectrophotometer. Results in terms of carbon monoxide were calculated by the use of a formula derived from the calibration curve and the chemical reactions involved. The method is sensitive, convenient (requiring the preparation of only one reagent, 1 per cent potassium iodide), and reliable for concentrations of carbon monoxide as low as 0.005 to 0.001 per cent. The range of applicability is 0.001 to approximately 0.2 per cent. Interfering substances (gasoline vapor, water, etc.) are effectively removed by the use of chromic acid, silica gel, and phosphorus pentoxide as the absorbing or "scouring" agents. As described, the method has an accuracy of  $\pm 10$  per cent in the range 0.001 to 0.010 per cent carbon monoxide. For analyses of carbon monoxide concentrations above 0.010 per cent and within the limits of usefulness of the apparatus (0.2 per cent) the accuracy of the method is increased to  $\pm 3$  to 5 per cent.

ONE of the standard methods and probably the most widely used quantitative procedure at present available for the determination of small amounts of carbon monoxide in air is the iodine pentoxide method. Numerous workers, including Edell (1), Sendroy (3), and Vandaveer and Gregg (4, 5) have suggested modifications or have added improvements to the method as originally introduced by de la Harpe and Reverdin (2).

In these methods the gas sample is usually first passed through chromic acid to remove volatile hydrocarbons, then through potassium hydroxide and phosphorus pentoxide to remove water vapor, and finally over dry solid iodine pentoxide at 150° to 160° C. to produce quantitatively the volatile products carbon dioxide and iodine. These are absorbed or collected in barium hydroxide in case carbon dioxide is measured, or in potassium iodide in case the iodine is to be determined. Iodometric measurement by titration with sodium thiosulfate, using starch as the indicator, has been generally adopted as the method of choice.

However, the necessity for frequent determinations in this laboratory of low (0.005 to 0.001 per cent) carbon monoxide concentrations disclosed several difficulties and disadvantages which made desirable some other means of measuring the iodine

liberated. The following may be mentioned specifically: (1) the very dilute (0.001N) sodium thiosulfate solutions used, because of a slow rate of decomposition, require periodic checks upon their concentration; (2) slight overtitration of the end point is a potential source of error; and (3) duplicate titrations on the same sample are sometimes impossible.

- ACKNOWLEDGMENTS
- The authors gratefully acknowledge the cooperation of V. Migrdichian of the American Cyanamid Company for his suggestions as to the sampling medium, and of W. P. Tyler, T. R. Steadman, and J. C. McCool of the B. F. Goodrich Company in the development of the analysis.

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## APPARATUS AND REAGENTS

Iodine pentoxide apparatus. Coleman photoelectric spectrophotometer, Model 10S. One per cent potassium iodide solution (Merck's C.P. granular potassium iodide is satisfactory). Pipets 10.0, 1.0, and 0.2 cc.

METHODS AND PROCEDURE

The above-mentioned difficulties could be eliminated, it was found, by the use of a spectrophotometric method of measuring the iodine concentration. For this purpose a Coleman double monochromator photoelectric spectrophotometer (Model 10S) having a range of 350 to 1000 millimicrons was used. Determinations of the spectral transmittance of iodine in per cent potassium iodide solution, using the calibration curve relating the percentage transmittance to logarithm of the iodine concentration in blanks and unknown samples were performed with this instrument.

The spectrophotometric method of measuring the amount of iodine in a 1 per cent potassium iodide solution requires no chemical reaction of the iodine but is dependent only upon the absorption of light by the iodine in solution. For the range of iodine concentrations used this absorption has been observed to follow Beer's law, in that the relationship between the logarithm of the percentage transmittance and the con-

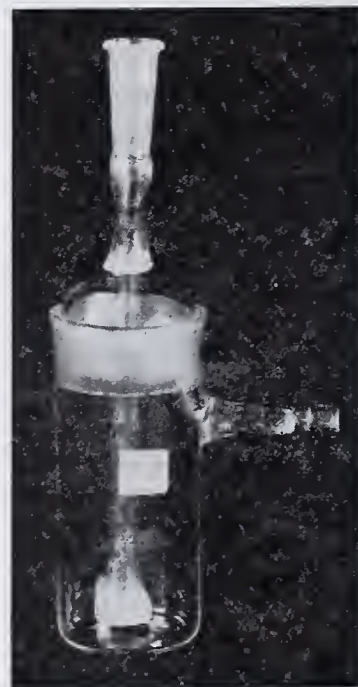


Figure 1. Absorption Tube



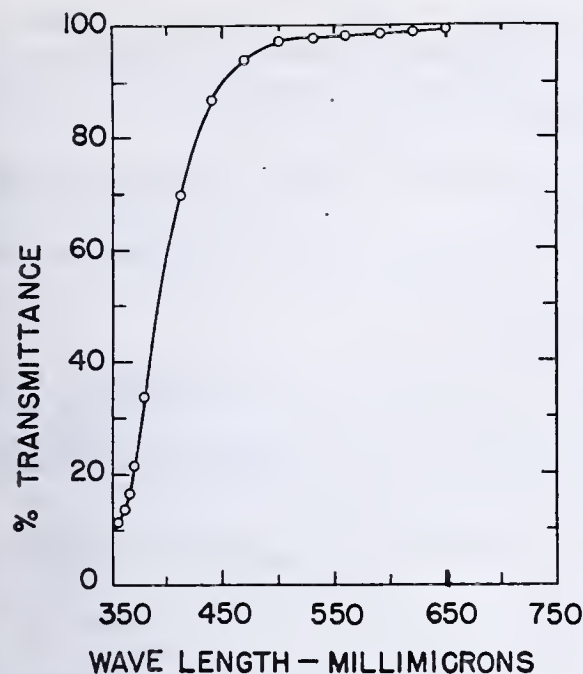


Figure 2. Spectral Transmission Curve of Iodine ( $5 \times 10^{-5}N$ )

In 1 per cent potassium iodide solution as determined with Coleman photoelectric spectrophotometer (Model 10S) using a 5-mm. slit. Cuvettes were circular tubes of 16.5-mm. diameter and comparison tube was a blank solution (10 cc.) of 1 per cent potassium iodide

centration of absorbing material (iodine in 1 per cent potassium iodide) is linear and the calibration curve is a straight line. This may be expressed as:

$$\text{Concentration} = K' \times \frac{\log T_0}{T}$$

where  $T_0$  equals the intensity of incident light,  $T$  equals the intensity of transmitted light (after passing through sample), and  $K'$  equals the calibration constant expressing the relationship between concentration of iodine in 1 per cent potassium iodide solution and the logarithm of the percentage transmittance. Absorption of light by iodine ( $5 \times 10^{-5}N$ ) in 1 per cent potassium iodide increases as the wave length of light used decreases into the ultraviolet. As a result of this observation all measurements were made at 350 millimicrons, the point of greatest absorption of the wave-length range available with this type spectrophotometer.

Gas samples are collected in calibrated metallic containers of approximately 1600- to 1700-cc. (STP) capacity; smaller 200- to 250-cc. (STP) glass tonometers may be used for sampling purposes, but give less satisfactory results. The samples are then passed through the iodine pentoxide apparatus by displacement with water, this method being preferable from the standpoint of safety, ease of adjustment, and convenience to the suction method usually employed. It was experimentally observed that the displacement of the 1600- to 1700-cc. sample in 60 minutes followed by a 30-minute flushing with nitrogen gas at the same rate of flow gave most accurate results. However, size of sample, its concentration, and degree of accuracy demanded are all factors which may modify the time necessary to complete an analysis.

For the collection of the liberated iodine the original Gomberg tube used routinely with the iodine pentoxide apparatus was replaced by an absorption tube designed and adapted by J. W. Heim of this laboratory for the present method of analysis (Figure 1). The iodine passes through the collecting tube, which extends almost to the bottom of the absorption vessel, and bubbles out through the sintered-glass filter at its end. The amount of iodide solution used (10 cc.) is sufficient, of course, to keep the filter well below the liquid level. This tube has marked advantages over the Gomberg type, in that its wide-mouthed ground-glass stopper permits ready access to the iodide solution for sampling at the completion of an analysis; it is easily washed out or cleansed; and quantitative results obtained with its use have proved it an efficient type of absorption vessel.

Blank analyses are carried out by filling calibrated containers with nitrogen (commercial), passing the gas through the apparatus, and flushing exactly as with unknown samples. From the amount of iodine measured the equivalent carbon monoxide is then calculated. Blank analyses are always performed prior to the analysis of unknown samples and the blank value in terms of percentage carbon monoxide is always subtracted from the unknown value to obtain the true or "corrected" carbon monoxide percentage concentration. The only reagent required for an analysis is the 1 per cent potassium iodide solution. This should be prepared from a pure grade of potassium iodide (since impure preparations readily liberate appreciable iodine) and should be renewed about once a week. The solution should be stored in a dark glass bottle.

## RESULTS

In Figure 2 the spectral transmittance curve of iodine ( $5 \times 10^{-5}N$ ) in 1 per cent iodide solution is presented. Maximum absorption of light by iodine ( $5 \times 10^{-5}N$ ) in 1 per cent potassium iodide solution occurs in the region of the ultraviolet. However, at 350 millimicrons (the lower limit of the Coleman instrument) the absorption is sufficiently great (90 per cent) to provide a means of measurement that is as sensitive and accurate as the routine sodium thiosulfate titration method. Both the titration method and that described here have been employed in this laboratory for determining carbon monoxide in air samples. However, the authors have found the titration method less satisfactory for concentrations of carbon monoxide below 0.005 per cent. Below this value the titration method was less convenient and more time-consuming than the spectrophotometric.

The relationship between the concentration of iodine over a wide concentration range and the logarithm of percentage transmittance is linear. The value of the calibration constant,  $K'$ , which expresses this relationship between concentration in equivalents per liter and logarithm of percentage transmittance, is  $5.1 \times 10^{-5}$ .

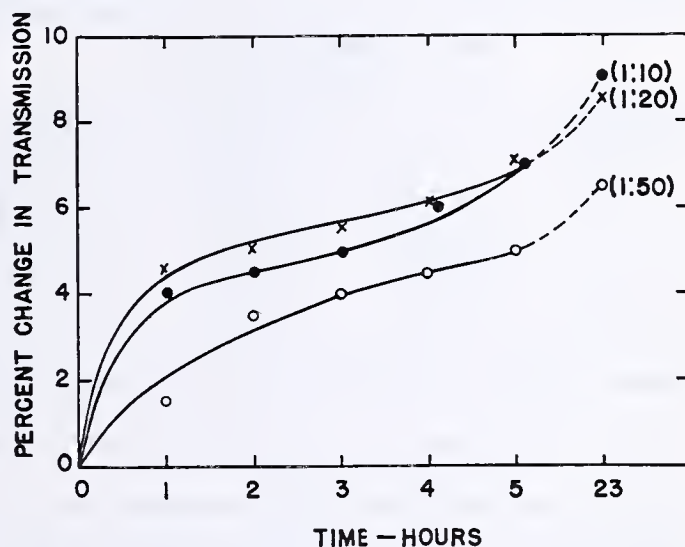


Figure 3. Changes in Percentage Transmission of Iodine in 1 Per Cent Potassium Iodide as a Function of Time

The change in the percentage light transmission of iodine in 1 per cent potassium iodide solution as a function of time is illustrated in Figure 3 and the quantitative effects of such changes are presented in Table I. Dilutions of 1 to 50 showed changes equivalent to only a 6 per cent decrease in the percentage of carbon monoxide after one hour. If spectrophotometric readings are made within 5 to 10 minutes following the completion of the sample run, the error due to any change in light transmission may be considered negligible.

Blank analyses using nitrogen gas (commercial) and performed as previously described gave fairly consistent values, the average being equivalent to a carbon monoxide concentration of 0.0004 per cent. These results are summarized in Table II.



Table I. Effect of Changes in Percentage Transmission of Iodine in 1 Per Cent Potassium Iodide Solution with Time

Original Concentration		1 Hour		5 Hours		23 Hours	
Dilution	CO	CO	Change	CO	Change	CO	Change
	%	%	%	%	%	%	%
1:10	0.0137	0.0120	-12.4	0.0109	-20.4	0.0103	-24.8
1:20	0.0136	0.0120	-11.8	0.0112	-17.6	0.0107	-21.4
1:50	0.0116	0.0109	- 6.0	0.0091	-21.6	0.0085	-26.8

Table II. Blank Analyses

[Nitrogen (Commercial) Gas Samples]

Date	Sample Volume Cc.	CO (as Iodine Equivalent) %
12/5/42	1660	0.0003
12/5/42	1610	0.0007
12/8/42	1630	0.0002
12/12/42	1590	0.0002
12/12/42	1600	0.0005
12/12/42	1580	0.0004
12/12/42	1640	0.0003
12/19/42	1630	0.0005
1/2/43	1630	0.0005
1/6/43	1650	0.0004

Av. 0.0004MD = (±) 0.0001

An opportunity to check the accuracy and sensitivity of this method was offered when known standard samples of carbon monoxide mixtures prepared and analyzed by the National Bureau of Standards, Washington, D. C., were sent to Wright Field for check analyses. The comparative results are presented in Table III. In general, values with the spectrophotometric method varied from 4 to 18 per cent below those reported by the Bureau of Standards, with results averaging 10 to 11 per cent lower. While the method is capable of detecting concentrations as low as 0.001 per cent, the limit of accuracy is approximately 5 to 10 per cent for concentrations below 0.010 per cent.

Table III. Carbon Monoxide

(Comparison of Bureau of Standards and Wright Field Analyses)

Bureau of Standards Analyses %	Date	Wright Field Analyses %	Difference %
0.0076	4/12/42	0.0070	-7.9
	4/12/42	0.0065	-14.5
	4/12/42	0.0068	-10.5
	4/12/42	0.0069	-9.1
		Av.	-10.5
0.0050	4/11/42	0.0041	-18.0
		0.0048	-4.0
		Av.	-11.0

The analysis of samples whose concentration is below 0.010 per cent requires consideration of several factors. As previously mentioned, rate of sample flow should be sufficiently slow to ensure complete oxidation of all carbon monoxide by the iodine pentoxide. For concentrations of 0.010 per cent and below total sampling periods of 80 to 90 minutes gave satisfactory results. Sample volume is another factor to be given consideration when accurate results are desired. Comparative results of analyses of a standard carbon monoxide mixture when using small and large sampling volumes are shown in Table IV. Accuracy and dependability of analysis are obviously increased when larger volumes are used, particularly when the concentration of carbon monoxide is below 0.010 per cent.

The photoelectric colorimeter used with a filter which transmits the greater portion of its light as nearly as possible within the region of the maximum absorption of iodine in 1 per cent potassium iodide solution—i.e., about 350 millimicrons—can be used if the spectrophotometer is not available. A calibration curve must be determined and the sensitivity of the photoelectric colorimetric procedure is somewhat less than that obtained with the spectrophotometer.

From the chemical reactions involved 1.0 ml. of 0.001N iodine solution is equivalent to 0.056 ml. of carbon monoxide measured at 0° and 760 mm. The volume of carbon monoxide at 0° and 760 mm. is then calculated as follows:

$$\% \text{ CO} = \frac{K' \times (0.056 \times 1000) \times 100 \times 10 \times (2 - \log \% T)}{\text{cc. of sample (STP)}}$$

where  $K'$  is  $5.1 \times 10^{-5}$  and 10 ml. of 1% potassium iodide is used.

substituting,

$$\% \text{ CO} = \frac{5.1 \times 10^{-5} \times (0.056 \times 1000) \times 1000 \times (2 - \log \% T)}{\text{cc. of sample (STP)}}$$

$$\text{or } \% \text{ CO} = \frac{5.1 \times 10^{-5} \times 56,000 \times (2 - \log \% T)}{\text{cc. of sample (STP)}}$$

$$\text{Finally } \% \text{ CO} = \frac{2.86 \times (2 - \log \% T)}{\text{cc. of sample (STP)}} \times \text{dilution used}$$

The value 2.86 is termed  $K$  in the final equation below and determined as indicated from both the calibration curve and the quantitative relationship between the concentration of carbon monoxide and iodine released in the oxidation reaction. The final general equation is then:

$$\% \text{ CO} = \frac{K \times (2 - \log \% T)}{\text{cc. of sample (STP)}} \times \text{dilution}$$

As an example of the use of the above equation in calculating results, the following analytical data are presented:

A 10-cc. portion of the 1 per cent potassium iodide was used to collect the iodine liberated when a gas sample of 2360 cc. (STP) was passed through the apparatus. A 1 to 100 dilution of iodine collected in the 10-cc. portion of the 1 per cent potassium iodide solution was made and when measured (at 350 millimicrons) with the spectrophotometer against a 10-cc. 1 per cent potassium iodide blank set at 100 per cent light transmission, gave a percentage light transmission of 44.5. The calculation, by substitution of the above data, is illustrated as follows:

$$\% \text{ CO} = \frac{2.86 \times (0.352)}{2360} \times 100 = 0.043$$

Table IV. Standard Carbon Monoxide Mixture

[Comparison of Analyses Using Small and Large Sample Volumes of Standard CO (0.012 per cent)]

Date	Gas Volume Cc.	CO Found %	Deviation %
10/23/42	264	0.011	+0.001
10/23/42	264	0.009	-0.001
10/23/42	264	0.013	+0.003
10/24/42	270	0.006	-0.004
10/24/42	270	0.010	0.000
10/24/42	270	0.006	-0.004
10/24/42	270	0.015	+0.005
		Av. 0.010	MD = (±) 0.001
10/26/42	1660	0.013	+0.002
10/26/42	1660	0.010	-0.001
10/26/42	1660	0.011	0.000
10/27/42	1660	0.012	+0.001
10/27/42	1660	0.011	0.000
10/27/42	1660	0.013	+0.002
10/30/42	1660	0.011	0.000
10/31/42	1660	0.011	0.000
11/13/42	1645	0.012	+0.001
		Av. 0.011	MD = (±) 0.001

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# Yeast Microbiological Methods for Determination of Vitamins

## Pantothenic Acid

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The yeast growth method for determination of pyridoxine is modified for the determination of pantothenic acid. The basal medium contains ammonium sulfate as a nitrogen source and in addition sufficient asparagine to prevent interference due to  $\beta$ -alanine. Extracts of substances to be assayed are prepared by aqueous extraction under pressure (15 pounds for 15 minutes) at pH 5.6 to 5.7, by

enzyme digestion at the same pH, or by enzyme digestion followed by aqueous extraction (15 pounds for 15 minutes). The choice of extraction method depends upon the substance, since some have pantothenate in a bound form whereas others do not. The results of assays of a number of representative substances compare favorably with results obtained by other methods.

THE yeast microbiological method recently described for the determination of pyridoxine (2) can, with certain modifications, be used to determine pantothenic acid. The yeast growth for activity of pantothenic acid was known before its need in animal nutrition was established (12). That the yeast method was not further developed as an assay method by the discoverers of pantothenic acid was due in part to the interference of  $\beta$ -alanine (8).  $\beta$ -Alanine is a structural part of the vitamin molecule but is itself without vitamin activity for higher animals, although under certain conditions it can replace pantothenic acid as a yeast growth factor. The inclusion of asparagine in the yeast growth medium tends to reduce the effect of  $\beta$ -alanine (13), but apparently it was not realized that the presence of sufficient asparagine further reduces the interference to an insignificant level. Asparagine does not, however, affect the activity of pantothenic acid under the conditions employed.

The assay method described here has been used by the authors some time and may possess certain advantages over the microbiological method of Pennington, Snell, and Williams (8). The method is rapid, 16 to 18 hours being allowed for yeast growth, and it is especially adapted for turbidimetric measurement of yeast growth with a photoelectric colorimeter. Furthermore, it offers an opportunity for checking assay results with a different type of microorganism.

### APPARATUS

The apparatus employed has previously been described (1, 2). The utility of the Evelyn photoelectric colorimeter was also studied. Using the test tubes usually supplied with the Evelyn and the 660 (red) filter, the absorption curves were found to be essentially the same as with the Lumetron instrument.

### SOLUTIONS

**SUGAR AND SALTS SOLUTION.** One liter contains 200 grams of D. dextrose (anhydrous), 2.2 grams of monopotassium phosphate, 1.7 grams of potassium chloride, 0.5 gram of magnesium sulfate ( $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ ), 0.5 gram of calcium chloride ( $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ ), 0.01 gram of manganese sulfate, and 0.01 gram of ferric chloride.

**POTASSIUM CITRATE BUFFER.** One liter contains 100 grams of potassium citrate ( $\text{K}_3\text{C}_6\text{H}_5\text{O}_7 \cdot \text{H}_2\text{O}$ ) and 20 grams of citric acid ( $\text{C}_6\text{H}_8\text{O}_7$ ).

**THIAMINE SOLUTION,** 10 micrograms per ml.

**PYRIDOXINE SOLUTION,** 10 micrograms per ml.

**INOSITOL SOLUTION,** 1 mg. per ml.

**BIOTIN SOLUTION.** S. M. A. Corp. biotin concentrate No. 100, diluted so that it contains approximately 0.8 microgram of biotin per ml. On one occasion this material was found to contain appreciable amounts of pantothenate and as a consequence a high blank, 15 to 20 per cent absorption, was obtained. The pantothenate was readily destroyed by alkaline heat treatment and the blank or zero value returned to the previous value (less than 10 per cent). Pure biotin was also tried when it recently

became available and the results indicate that it may be substituted for the crude concentrate.

**AMMONIUM SULFATE SOLUTION,** 150 mg. per ml.

**ASPARAGINE SOLUTION.** One hundred milliliters contain 3 grams of L-asparagine. Heating to 100° C. is necessary to dissolve the asparagine.

The solutions are sterilized by heating in flowing steam for 30 minutes on three consecutive days, and may then be stored at room temperature until used. The pyridoxine solution is carefully protected from light.

**PANTOTHENATE STANDARD SOLUTION.** D-Calcium pantothenate (synthetic) is used as a primary standard. A freshly dissolved solution containing 1 mg. per ml. is kept in the refrigerator and used as a working standard for not more than 2 to 3 weeks. Immediately before use a portion of the working standard is diluted with distilled water, to contain 0.1 microgram (100 millimicrograms) per ml.

### YEAST INOCULUM

Fleischmann culture 4228, a strain of *Saccharomyces carlsbergensis*, is grown on malt agar slants (Difco) for 24 hours at 30° and then may be stored in the refrigerator for not more than one month. The day before an assay run, a fresh transfer is made and incubated at 30°. Yeast is transferred from this slant to a tube of sterile saline until the light absorption indicates that the concentration is 3 mg. per ml. The calibration (50 per cent absorption with the authors' instrument) is based on a known suspension of moist baker's yeast. The absolute quantity of yeast in the inoculum is not critical and hence this approximation is satisfactory. A 10-ml. aliquot of this suspension is added to 90 ml. of sterile saline contained in an Erlenmeyer flask. The final suspension contains 0.3 mg. of moist yeast per ml. and is ready for use.

### PREPARATION OF SAMPLES FOR ASSAY

Kuhn and Wendt (7) have reported that pantothenate (filtrate factor) may occur as part of a nondialyzable complex of high molecular weight. The pantothenate content of tissues and yeast, as measured by microbiological methods, is increased by autolysis or enzyme digestion and the increase appears to be due to a decomposition of the complex. Pantothenate is a relatively unstable compound, being readily hydrolyzed by either acid or base. Consequently it is desirable to prepare extracts for assay by the mildest and most direct means. In general, there are three extraction methods available:

1. Enzyme digestion by clarase or other suitable enzyme preparations. Autolysis (self-digestion) may be considered a form of enzyme digestion but is inapplicable to most substances and uncertain with some tissues.

2. Water extraction at high temperature (autoclave) and at the most stable pH range.

3. Enzyme digestion followed by water extraction at high temperature.



Table I. Typical Protocol

To each tube are added 5 ml. of basal pantothenic acid-free medium plus ingredients noted below. After the 10-minute heat treatment 1 ml. of suspension (0.3 mg. of moist yeast) is added to each. The tubes are then shaken at 30° for 16 hours, the turbidity is measured, and the tubes are returned to the incubator for 2 hours and measured again.

No.	H <sub>2</sub> O	Added		16 Hours			18 Hours			Average
				Absorption	Pantothenate	Pantothenate	Absorption	Pantothenate	Pantothenate	
	Ml.	Ml.		%	Mγ/tube	γ/g. or γ/ml.	%	Mγ/tube	γ/g. or γ/ml.	γ/ml.
1	4.0	0		6	...	...	7	...	...	...
2	3.5	0.5	50 Mγ of calcium pantothenate <sup>a</sup>	16	...	...	21	...	...	...
3	3.0	1	100 Same	29	...	...	37	...	...	...
4	2.5	1.5	150 Same	41.5	...	...	48	...	...	...
5	2.0	2	200 Same	48.5	...	...	55	...	...	...
6	1.0	3	300 Same	59.5	...	...	66	...	...	...
7	0	4	400 Same	66.5	...	...	73	...	...	...
8	3	1	0.025 ml. whole urine <sup>b</sup>	21	70	2.8	28.5	72	2.9	28.5
9	2	2	...	31	130	2.6	36.5	130	2.6	36.5
10	1	3	...	48	195	2.6	55.5	203	2.8	55.5
11	0	4	...	54.5	250	2.5	61.5	250	2.5	61.5
12	3	1	0.1 mg. yeast extract (dry) <sup>c</sup>	17.5	57	570	22.5	55	550	550
13	2	2	...	31.5	110	550	40	112	560	560
14	1	3	...	43.0	165	550	53	165	550	550
15	0	4	...	50.5	215	538	57	212	530	530
16	3	1	0.5 mg. dry 200-B yeast <sup>d</sup>	22.5	75	150	29	75	150	150
17	2	2	...	41.5	155	155	48.5	155	155	155
18	1	3	...	56	265	177	61.5	250	166	166
19	0	4	...	63	355	173	67.5	317	159	159
20	3	1	10 mg. wheat-hard winter No. 2 <sup>e</sup>	25.5	87	8.7	32.5	86	8.6	8.6
21	2	2	...	41.5	157	7.9	49.5	160	8.0	8.0
22	1	3	...	54.5	250	8.3	60	235	7.8	7.8
23	0	4	...	62.5	350	8.8	67.5	320	8.0	8.0

<sup>a</sup> Calcium pantothenate, prepared from 1 mg. per ml. of refrigerated solution.  
<sup>b</sup> No treatment, preserved at pH 5.6 for 24 hours, 10 ml. diluted to 400 ml.  
<sup>c</sup> Digested with clarase for 2 days at 45° C., 100 mg. diluted to 1 liter.  
<sup>d</sup> Digested with clarase for 2 days at 45° C., 100 mg. diluted to 200 ml., solution centrifuged until clear.  
<sup>e</sup> Water extraction, autoclaved 15 minutes at 16 pounds pressure, 1 gram diluted to 100 ml.

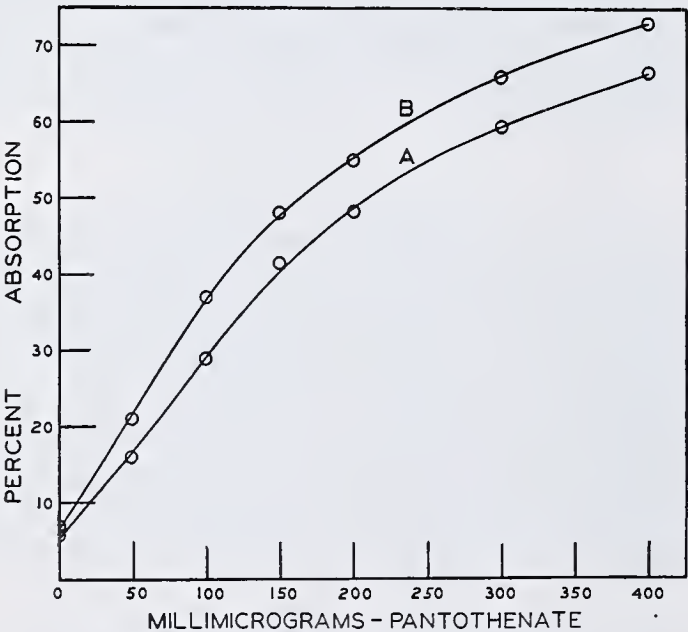


Figure 1. Reference Curve  
A. 16 hours      B. 18 hours

The third method has been found necessary with certain materials—e.g., fresh green peas—because of the presence of a substance inhibitory to yeast growth which, however, is inactivated by a short treatment in the autoclave.

**ENZYME DIGESTION.** Insoluble substances should be powdered or dispersed in water with a Waring Blendor or its equivalent. Weigh or measure a portion of the unknown estimated to contain 10 to 20 micrograms of pantothenate into a 40-ml. test tube graduated at 10 and 20 ml. Add 1.0 ml. of the buffer and sufficient water to make the volume to 10 ml. Heat in flowing steam for 5 minutes, cool, and add a weighed amount of clarase roughly equal to the dry weight of the sample. Dissolve by gentle shaking and after adjusting the volume to 20 ml. add 0.5 ml. of benzene. Cork securely and incubate at 45° for 2 days or 37.5° for 3 days. Make the volume to 200 ml. with water. With materials of high potency it is not convenient to weigh out

an amount containing only 10 to 20 micrograms; hence 100 are weighed out and after digestion a greater dilution is made. The solutions are centrifuged, if necessary, to obtain a clear extract.

**WATER EXTRACTION (HIGH TEMPERATURE).** Some cereal products do not appear to require enzyme digestion (9, 10)—e.g., wheat and wheat products—and for these water extraction may be used. Suspend an amount of sample estimated to contain 5 to 10 mg. of pantothenate—e.g., 1 gram of wheat—in 80 ml. of water, add 1 ml. of buffer, and adjust the pH to 5.6 to 5.7, using dilute sodium hydroxide or sulfuric acid. Heat the suspensions in an autoclave at 7 kg. (15 pounds) for 15 minutes, cool, and dilute to 100 ml. This treatment does not always yield a clear extract, even after centrifuging. A short incubation at 45° for 15 minutes after the addition of a knife point of clarase will usually produce flocculation and a clear supernatant fluid.

**ENZYME DIGESTION FOLLOWED BY WATER EXTRACTION.** Proceed exactly as in the simple enzyme digestion but rinse the contents of the test tube into a flask with 60 ml. of water, check the pH and adjust, if necessary, to 5.6 to 5.7, and then heat at 15 pounds for 15 minutes. Cool and dilute as usual.

METHOD

Five milliliters of basal pantothenate-free medium plus a suspension of the unknown or an aliquot of the pantothenate standard solution are placed in a series of test tubes together with sufficient water to make the volume in each tube 9 ml. The tubes are plugged with cotton, steamed for 10 minutes, cooled, and inoculated with 1 ml. each of the yeast inoculum. The tubes are shaken at 30° C. and the yeast growth is estimated at 16 and 18 hours by turbidimetric measurements made directly on the tubes with the photoelectric colorimeter. Each assay run includes a series of tubes which are used to construct the reference curve. This series is made with the following levels: 0, 50, 100, 150, 200, 300, and 400 millimicrograms per tube. For assay runs containing more than 25 tubes two reference series are included, one at the beginning and one at the end of the run, and the results of two are averaged to construct the reference curve.

The basal medium for 20 assay tubes is prepared by mixing stock solutions in the following proportions: sugar and solution, 50 ml.; potassium citrate buffer, 10 ml.; inositol solution, 5 ml.; ammonium sulfate solution, 5 ml.; thiamine solution, 5 ml.; pyridoxine solution, 5 ml.; biotin solution, 5 ml.; asparagine solution, 12.5 ml.; and water to 100 ml.

It is not essential to prepare this medium fresh for each assay. Larger batches may be prepared and stored at a temperature a few degrees below 0° for as long as 3 months with no observable effects upon the assay run.



The protocol of a typical assay run in which representative materials were assayed is shown in Table I and the reference curve is given in Figure 1. In practice this curve is plotted on ordinary graph paper and values for the unknown are obtained by interpolation. The estimated potency is an average of the value at each assay level and at both 16 and 18 hours. The average deviation from the mean is about 4 per cent. If more than two of the eight values obtained for each assay deviate from the mean by more than 10 per cent, the assay is usually repeated.

Results are reported as pantothenate content and are based on *d*-calcium pantothenate as the primary standard without conversion to the equivalent weight of free acid. There seems to be no precedent for this in the use of thiamine hydrochloride and pyridoxine hydrochloride equivalents without conversion to the equivalent weight of free base.

**Table II. Determination of Pantothenate in Presence of Its Hydrolytic Products**

Composition of Mixture		Assay	
Completely hydrolyzed calcium pantothenate	Calcium pantothenate	Calcium pantothenate (by assay)	Recovery of added calcium pantothenate
Mg.	Mg.	Mg.	%
0	1.0	1.02	102
0.25	0.75	0.70	93
0.50	0.50	0.50	100
0.70	0.30	0.29	97
0.80	0.20	0.19	95
0.90	0.10	0.10	100
0.95	0.05	0.055	110
1.00	0.00	<0.01	...

## RESULTS

**PANTOTHENATE ESTIMATION IN THE PRESENCE OF  $\beta$ -ALANINE.** Since  $\beta$ -alanine is a potential interfering substance in this assay it is desirable to determine the limits of the interference. There is no evidence that  $\beta$ -alanine occurs as such in nature except as a degradation product of pantothenate. Consequently, the influence of  $\beta$ -alanine on the assay was studied by analyzing a series of mixtures representing pantothenate in various stages of hydrolysis.

A solution of completely hydrolyzed pantothenate was prepared by heating 10 mg. of the calcium salt dissolved in 10 ml. of 1N and 4N sodium hydroxide, respectively, at 15 pounds pressure for 2 hours and then cooling and neutralizing. The pantothenate remaining was determined after adding a known amount of pantothenate to the solution. The assay showed 1.5 per cent of the pantothenate left in the 1N treated solution and less than 1 per cent in the 4N treated solution.

In order to determine the amount of  $\beta$ -alanine produced by the hydrolysis the basal medium was modified by the omission of paragine, the inclusion of *L*-leucine, and the substitution of biotin for the crude which contains  $\beta$ -alanine. A reference series of growth tests with increasing amounts of  $\beta$ -alanine then supplied data for a reference curve, which was used to determine the  $\beta$ -alanine content of the hydrolyzates. Theoretically each 10 parts of calcium pantothenate should yield 37.3 of  $\beta$ -alanine. The 1N solution assayed 32.5 parts or 87 per cent and the 4N solution 30.25 parts or 81 per cent. Apparently a portion of the  $\beta$ -alanine is destroyed in the course of the hydrolysis.

Assuming that the 4N solution represented "completely" hydrolyzed pantothenate, the experiment described in Table II was made. The hydrolyzate was mixed in various proportions with freshly dissolved pantothenate and the mixtures were assayed as unknowns. The results show that little interference from  $\beta$ -alanine is to be expected until 95 per cent or more of the pantothenate has been destroyed.

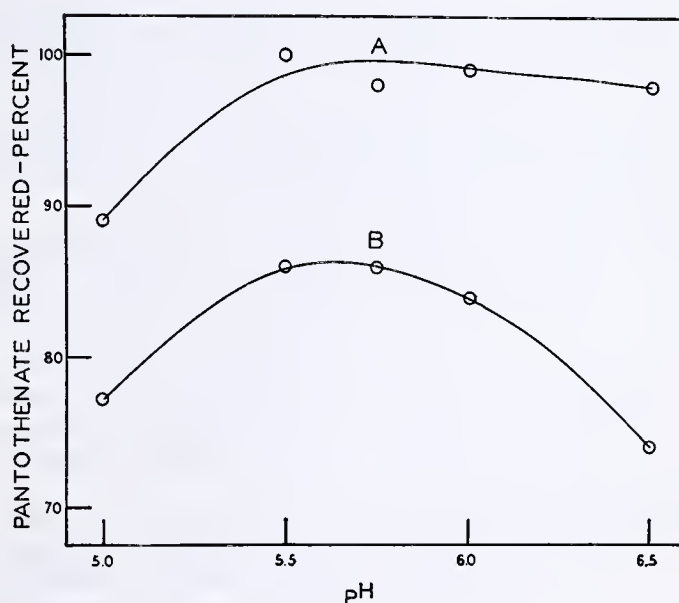
The ease with which the present method may be converted to a method for  $\beta$ -alanine determination suggests that it may be useful in determining the nature of the loss of pantothenate activity in the processing of vitamin concentrates.

**INFLUENCE OF pH ON THE STABILITY OF PANTOTHENATE.** Since pantothenate is readily hydrolyzed by either acid or alkali, it is desirable to establish the pH range of maximum stability, so that extracts for assay may be made with a minimum of loss.

Solutions of pantothenate were heated at 7 kg. (15 pounds) pressure for 15 minutes and at 9 kg. (20 pounds) for 1 hour. The heating at 20 pounds was used to accentuate the destruction; the milder heating is used in the assay. The solutions contained in 1 liter: 1 mg. of calcium pantothenate, 10 ml. of potassium citrate buffer, and enough citric acid to adjust the pH to 5.0. Potassium hydroxide (30 per cent) was added to adjust the pH to various levels and aliquots of the solution were removed at each level. After heating and cooling, the solutions were diluted to correspond to a concentration of 100 millimicrograms per ml. and assayed by the usual procedure.

As can be seen from Figure 2, the most stable region is between pH 5.5 and 6.0. In practice the pH is adjusted to between 5.6 and 5.7 in the extraction procedures. Although a suspension of whole wheat had a pH of 6.4, extraction without lowering the pH showed no significant loss. Relatively large quantities of organic matter may have a protective action on pantothenate. It is desirable, however, to adjust the pH to the most stable range in routine analysis, unless investigation shows it to be unnecessary. Clarification of aqueous extracts of starchy substances by the short clarase method proceeds best at pH 5.6 to 5.7.

**ENZYMATIC DIGESTION.** Early pantothenate assays of certain substances by the microbiological methods did not agree with chick assays (9). The discrepancy is now believed to be due to the existence of a bound form of pantothenate available to the chick but not to the microorganism. Digestion with various enzyme preparations liberates the bound pantothenate and tends to bring the assay results by the two methods into somewhat better agreement. Waisman and Elvehjem (11) found pancreaticin satisfactory, Cheldelin *et al.* (3) prefer takadiastase, whereas Strong, Feeney, and Earle (9) recommend clarase. The authors have compared clarase with a few other preparations and find it satisfactory and furthermore relatively low in color and in pantothenate content. The product is labeled "Diastase, Vera, highly concentrated 'Clarase'", and may be obtained from Eimer and Amend, New York. The pantothenate content of this preparation varies between 2 and 4 micrograms per gram. When clarase treatment is used, the results of the assay must be corrected for the pantothenate content of the enzyme.



**Figure 2. Influence of pH on Stability of Pantothenate**

A. 15 pounds for 15 minutes  
B. 20 pounds for 1 hour

The enzyme is active in the pH range of maximum stability of pantothenate. Maximum liberation of bound pantothenate was obtained by digestion for 3 days at 37° C. or 2 days at 45° C. Higher temperatures did not appear to offer any advantage. It is essential to maintain an excess of benzene or toluene in the digestion mixture during incubation.



Table III. Alkaline Hydrolysis of Pantothenate

Sample	Original Pantothenate Content $\gamma/g.$	Residual Pantothenate $\gamma/g.$	Destruction %
Yeast extract	509	2.5	99.5
	509	5.0	99.0
Dried yeast	164	10.0	94.0
Urine	3.2 $\gamma/ml.$	0.06 $\gamma/ml.$	98.4
Calcium pantothenate	1 mg.	10.0 $\gamma/mg.$	99

Table IV. Recovery of Pantothenate

	Total $\gamma/g.$	Added $\gamma/g.$	Found $\gamma/g.$	Recovered $\gamma/g.$	Recovery %
Wheat	8.3	8.0	17.4	9.1	116
	8.3	8.0	16.4	8.1	101
Yeast extract	550	500	1053	503	101
	550	500	1080	530	106
Dried yeast	164	200	371	207	104
	164	200	357	193	97
					Av. 103.6

**HYDROLYSIS OF PANTOTHENATE.** The relative ease with which pantothenate activity is destroyed by hydrolysis suggests an added test of the specificity of the assay method. Quantitative destruction of the pantothenate activity by alkaline hydrolysis would indicate the absence of an alkali-stable, nonspecific growth factor in the preparation under study. Similarly, acid hydrolysis would indicate the absence of an acid-stable factor.

Yeast extract (100 mg.) and dry yeast (100 mg.) were subjected to clarase digestion and then 10*N* sodium hydroxide was added to make the final concentration 1*N*. Urine and calcium pantothenate were prepared in similar fashion but without digestion. All were heated at 20 pounds for 2 hours, cooled and neutralized, and then assayed for pantothenate after superimposing a known quantity of pantothenate on the test. The results are shown in Table III. It is apparent that essentially all the activity is destroyed by alkaline hydrolysis. Similar results were obtained by acid hydrolysis.

**RECOVERY OF ADDED PANTOTHENATE.** The recovery of pantothenate which has been added to the unknown is a necessary test of the specificity of an assay method of this sort. Table IV gives the results obtained with wheat, yeast extract, and dried yeast, when the pantothenate was added at the beginning of the extraction. The calculated recovery is based upon the added pantothenate and the average recovery of 103.6 per cent is considered satisfactory, since each estimate is based upon two assays.

**ASSAY OF MISCELLANEOUS SUBSTANCES.** Table V gives the results of assays on a number of representative materials. These assays are in essential agreement with those appearing in the literature if allowance is made for the fact that many early microbiological assays were made without enzymic digestion. Wheat and wheat derivatives appear to give maximum values with simple aqueous extraction, but yellow corn requires enzymic digestion. It would thus appear difficult to generalize about the best extraction method for cereals.

Citrus fruits contain some of the bound pantothenate. Meats, animal tissues and extracts, and yeast products have a high proportion of bound pantothenate and must always be subjected to enzymic digestion. Milk and urine do not appear to contain any significant proportion of bound pantothenate. Enzyme digestion of fresh whole milk does not yield results as high as aqueous extraction. There are indications that fresh milk may have an inhibitory substance which is destroyed by heat. Most fresh vegetables contain bound pantothenate. Fresh green peas contain a substance which is markedly inhibitory to yeast growth but which appears to be completely destroyed when the autoclave method of extraction is employed. Simple enzymic digestion is therefore inadequate and the authors follow the enzyme treatment with autoclaving, when assaying fresh vegetables and fruits.

Table V. Pantothenate Content of Various Substances

Description	Pantothenate Determined Water extraction (autoclave) $\gamma/g.$	Clarase digestion $\gamma/g.$	Pantothenate Literature Value $\gamma/g.$
Cereals			
Whole wheat A	8.9	8.4	12 (4), 12.8 (10)
Whole wheat B	8.3		8.3 (9), 11.2 <sup>a</sup> (6)
White flour (patent)	4.0	3.9	3.5 (4), 5.7 (10)
Whole wheat bread (air-dry)	8.9		8.8 (4)
White bread (air-dry)	3.8		6.9 (4)
Yellow corn	4.2	9.3	9.0 (9)
Citrus fruits			
Grapefruit (fresh)	2.7	3.7 <sup>b</sup>	2.9 (4)
Oranges (fresh)	1.9	3.3 <sup>b</sup>	3.4 (4)
Clarase			
A	....	1.9	.....
B	....	4.0	.....
C	....	3.3	.....
Meats			
Pork muscle (fresh)	....	8.5	4.7, 5.8 (4)
Pork liver (fresh)	....	66.0	50 (11)
Beef muscle (fresh)	....	8.2	10 (9), 9.8 <sup>a</sup> (5)
Beef liver (fresh)	....	59.0	76 (4), 61.5 (9)
Liver concentrate No. 20 (dry)	....	472	.....
	$\gamma/ml.$ or $\gamma/g.$	$\gamma/ml.$ or $\gamma/g.$	$\gamma/ml.$ or $\gamma/g.$
Milk			
Pasteurized A	3.2	....	4.0 (9)
Pasteurized B	3.3	1.8, 2.4 <sup>b</sup>	.....
Dried skim milk	43.0	46.0	44, 47 (9)
Milk (whey) <sup>c</sup>	3.2	....	.....
	$\gamma/day$	$\gamma/day$	$\gamma/day$
Urine, normal 24-hour excretion <sup>d</sup>			
Subject A	3410	3250	3000-5000 (9)
Subject A	4400	....	.....
Subject B	4400	....	.....
Vegetables (fresh)			
Tomatoes	2.0	4.5 <sup>b</sup>	3.7 (4)
Cabbage	2.1	2.5 <sup>b</sup>	1.8 (4)
Beets	1.3	1.6 <sup>b</sup>	1.1 (4)
Carrots	2.3	3.1 <sup>b</sup>	2.5 (4)
Green peas	3.8	3.9 <sup>b</sup>	3.8 (4)
Potatoes	2.1	2.5 <sup>b</sup>	3.2 (4)
Yeast			
Brewers' (dry)	....	100	22 <sup>e</sup> , 86 <sup>e</sup> (9)
200-B (dry)	88	164	.....
Yeast extract (dry)	319	500	240 <sup>e</sup> (9), 266 <sup>e</sup> (8)
Autoclaved (dry)	....	48	.....

<sup>a</sup> Chick assay.

<sup>b</sup> Clarase digestion followed by autoclaving with water.

<sup>c</sup> Casein coagulated with mineral acid and supernatant fluid neutralized and autoclaved for assay.

<sup>d</sup> pH of urine adjusted and assayed without further treatment.

<sup>e</sup> No enzymic digestion.

The specificity of the present method is supported by a number of observations: The values estimated at the various testing levels and at 16 and 18 hours show no significant drift, recovery of added pantothenate is virtually quantitative, the potential interference of  $\beta$ -alanine has been eliminated, the pantothenate activity of extracts prepared for assay may be destroyed by alkaline or acid hydrolysis, and the results obtained are in substantial agreement with reported values obtained by tests which employ other microorganisms, and also in a limited number of cases with the results of the chick assay method.

In order to obtain a measure of the reproducibility of the method, a carefully refrigerated sample of dried yeast was assayed ten times over a period of 3 months. The complete assay including clarase digestion was performed each time. The mean of the ten assays was 166.3 micrograms of pantothenate per gram with an average deviation of 3.2 per cent and a standard deviation of 3.8 per cent.

#### SUMMARY

A yeast microbiological method for the determination of pantothenate is described. Specificity of response to pantothenate in the presence of  $\beta$ -alanine is obtained by the inclusion of a relatively large proportion of asparagine in the medium in addition to ammonium sulfate. The yeast is grown in test tubes which are shaken at 30° C. for 16 to 18 hours. Yeast growth is estimated with the aid of a photoelectric colorimeter. Methods of extraction of the vitamin have been studied and recovery experiments are described. Extracts of substances to be assayed are prepared by aqueous extraction under pressure (15 pounds for 15 minutes).



pH 5.6 to 5.7, by enzyme digestion at the same pH, or by enzyme digestion followed by aqueous extraction (15 pounds for 15 minutes).

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# Polarographic Determination of Copper, Lead, and Cadmium in High-Purity Zinc Alloys

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A study has been made on the application of the polarographic method of analysis in determining trace elements (down to  $1 \times 10^{-4}$  per cent) found in zinc-base die casting alloys. Trace amounts of lead, cadmium, and tin cause intergranular corrosion which results in a serious weakening of the alloy. A polarographic procedure has been developed for the direct determination of copper, cadmium, and lead in these alloys. The samples are dissolved in hydrochloric and nitric acids, evaporated to near dryness, redissolved, treated with hydroxylamine hydrochloride, and finally diluted to volume. The solution is then electrolyzed cathodically over a range of approximately 0.8

volt to obtain waves for copper, lead, and cadmium. Using an 8-gram sample in 50 ml. of solution, these elements can be determined with a precision of  $\pm 1 \times 10^{-4}$  per cent of the sample weight. National Bureau of Standards zinc samples have been analyzed using the above procedure and the results found to agree very well with the certificate value. Samples of high-purity zinc and zinc alloys have been analyzed without difficulty. Nineteen elements have been considered from the standpoint of possible interference. The results indicate that trace amounts of copper, cadmium, and lead can be determined polarographically with high precision and accuracy.

METALLURGISTS have found in recent years that traces of lead, cadmium, and tin in zinc-base die-casting alloys tend to cause intergranular corrosion which results in a serious weakening of the alloy. For this reason, specifications for the manufacture of these alloys are very rigid, often requiring that lead shall not exceed 0.003 per cent, cadmium 0.003 per cent, and tin 0.001 per cent. The purpose of this investigation was to develop a system of analysis for zinc die-casting alloys of the Mazak type in which the polarograph could be used with advantage to determine trace amounts down to  $10^{-4}$  per cent with high precision and accuracy.

The determination of trace quantities by wet methods of analysis is exceedingly difficult. It is usually necessary to use large samples (100 grams or more), and to make repeated time-consuming separations. The polarographic method, on the other hand, is particularly suited to trace amounts, the very nature of which greatly reduces the necessity for making separations, and which compares very favorably with the spectrochemical method with respect to the limits of determination possible. A polarographic determination, where applicable, is considerably cheaper than a spectrochemical determination, and can often be carried out more rapidly.

Heyrovský (4) in a review of the applications of polarography noted that it is possible to determine lead and cadmium in zinc, and gave curves for a 0.5-gram sample in 5 ml. of hydrochloric acid, in which the concentration of lead is 0.0050 per cent and cadmium is 0.0037 per cent. Terui (11) determined lead and cadmium to the nearest  $1 \times 10^{-3}$  per cent by dissolving 8 grams of zinc in 70 ml. of 5N hydrochloric acid with a few drops of nitric acid and evaporating to 50 ml. Ensslin (3) reported a polarographic method for lead and cadmium in pure zinc, in which the zinc was dissolved in nitric acid and the resulting solution combined with different base solutions. The lead and cadmium were determined separately; the lead with an accuracy of 20 per

cent of the total amount present, from  $3 \times 10^{-3}$  to  $5 \times 10^{-4}$  per cent on what would correspond to a 100-gram sample in 1 liter of solution. Krossin (8) applied the polarograph to the analysis of copper- and aluminum-bearing zinc alloys for lead and bismuth by means of precipitation with sodium sulfide. Seith and Esche (9) determined lead, cadmium, bismuth, thallium, and tin in zinc by the polarographic method. The lead, cadmium, and bismuth were determined simultaneously by treating a 5-gram sample with hydrochloric acid and diluting to 25 ml. before electrolysis at 28° C. The thallium and tin were determined by difference from the sum of cadmium and thallium and lead and tin, respectively. Results are reported to the nearest  $1 \times 10^{-3}$  per cent except for tin, which is limited to  $1.5 \times 10^{-3}$  per cent. Hohn (5) in a review of polarographic methods of analysis outlined a method for copper, lead, and cadmium in zinc, but made no mention of its accuracy or precision.

In the work reviewed above, only one paper deals with the determination of impurities in zinc alloys, and this involves an objectionable sulfide separation with its attendant errors. The present investigation was undertaken in an effort to develop a method for the direct determination of copper, lead, and cadmium in high-purity zinc-base die-casting alloys of the Mazak type with special emphasis on accuracy and precision in the region of  $10^{-4}$  per cent of the sample weight.

## APPARATUS AND REAGENTS

The preliminary studies were made with a Leeds & Northrup Electro-Chemograph, and the work was concluded with a Heyrovský polarograph Model XI (E. H. Sargent and Co.). The same capillary was used throughout the investigation. The capillary constant in 2.5M zinc chloride was found to be  $1.37 \text{ mg.}^{2/3} \text{ sec.}^{-1/2}$  when  $h = 36.5 \text{ cm.}$ ,  $t = 3.3 \text{ seconds}$ , and temperature =  $25 \pm 0.5^\circ \text{ C.}$  When the curves showed irregularities traceable to fluctuations in the drop time, the capillary was cleaned with concentrated nitric acid as directed by Kolthoff and Lingane (7). The pressure on the dropping electrode was maintained by using the Leeds & Northrup electrode assembly in con-



junction with a large flask to serve as a pressure regulator. This kept the pressure constant to within  $\pm 0.5$  mm. of mercury over periods of not less than 45 minutes. An ordinary electrolysis cell with an internal anode was used throughout these experiments. The step heights were measured by the slope intercept method—i.e., straight lines were drawn along the principal slopes of the curve and the vertical distances between the points of intersection of the extensions of these lines were measured with a millimeter scale (1) (see Figure 1). All work was carried out at  $25 \pm 0.5^\circ \text{C}$ .

Most commercial reagents contain traces of various elements, and it was found necessary to check the purity of the reagents used under the conditions existing in the procedure outlined below. It was not possible to procure zinc metal of such purity that no steps were obtained under the operating conditions. (The word "step" is intended to describe the increase in current caused by the discharge of an ion, 7.)

A sample of zinc was made uniform by reducing the sample to shavings and mixing. A portion of this sample was analyzed polarographically, using the procedure outlined, without addition of metal ions. This gave the residual current for the ensuing determinations.

**CONCENTRATED HYDROCHLORIC ACID** (sp. gr. 1.19). One hundred milliliters of concentrated hydrochloric acid were evaporated almost to dryness in a 125-ml. Pyrex beaker. An 8-gram sample of zinc was then added to this residual liquid and the whole carried through the procedure for analysis. After subtracting the residual current due to the zinc, it was found that for the 32 ml. of hydrochloric acid required, a correction of  $5 \times 10^{-5}$  per cent of cadmium and  $5 \times 10^{-5}$  per cent for copper would have to be applied. No trace of lead was found.

**CONCENTRATED NITRIC ACID** (sp. gr. 1.42). One hundred milliliters of concentrated nitric acid were treated in a manner similar to that used for the hydrochloric acid. No traces of copper or lead were found, but cadmium corresponding to 0.00015 per cent in an 8-gram zinc sample was detected. Inasmuch as the amounts of nitric acid used were seldom in excess of 10 ml., this amount of cadmium was considered negligible for the present purpose.

**HYDROXYLAMINE HYDROCHLORIDE** (2N). Ten milliliters of 2N hydroxylamine hydrochloride were evaporated almost to dryness and treated as was the hydrochloric acid. No traces of copper, lead, or cadmium were found.

**GELATIN SOLUTION** (0.2 per cent aqueous). One gram of gelatin was ashed in a porcelain crucible and the residue taken up in a few milliliters of concentrated hydrochloric acid. The contents of the crucible were then added to a zinc sample as for hydrochloric acid. Copper corresponding to  $3 \times 10^{-4}$  per cent and cadmium to  $1 \times 10^{-4}$  per cent in an 8-gram zinc sample were found. Since only 2.5 ml. of the 0.2 per cent solution are used in an analysis, these impurities were considered negligible.

**DISTILLED WATER.** Five hundred milliliters of water were evaporated to dryness and treated as for hydrochloric acid. No detectable amounts of copper, lead, or cadmium were found.

Using the procedure described below, it was found that the over-all effect of impurities in the reagents amounts to copper 0.00005 per cent, lead 0.00000 per cent, and cadmium 0.00005 per cent. These amounts may be neglected for most purposes.

The standard solutions of copper, lead, and cadmium, required for the calibration of the capillary were prepared by diluting stock solutions.

**STANDARD STOCK SOLUTION FOR COPPER.** A 0.2M solution of cupric nitrate was prepared by dissolving 12.714 grams of electrolytic copper in dilute nitric acid and diluting to 1 liter. This solution was analyzed by slow deposition, and found to be  $0.1982 \pm 0.0002M$  (average of four determinations).

**STANDARD STOCK SOLUTION FOR CADMIUM.** A 0.2M solution of cadmium nitrate was prepared by dissolving 22.496 grams of c.p. cadmium in dilute nitric acid and diluting 1 liter. This solution was analyzed by electrical deposition and found to be  $0.1996 \pm 0.0004M$  (average of three determinations).

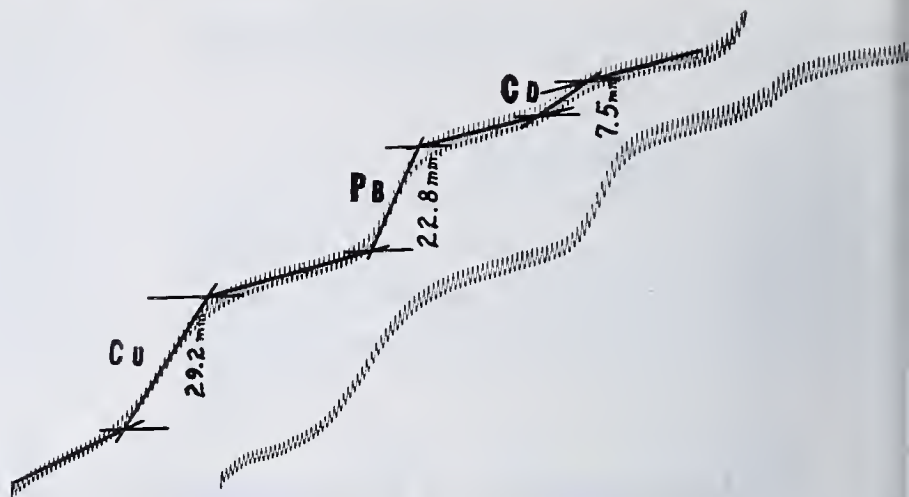


Figure 1. Typical Polarogram and Method of Measuring Wave Heights

**STANDARD STOCK SOLUTION FOR LEAD.** A 0.2M solution of lead nitrate was prepared by dissolving 41.458 grams of c.p. lead in dilute nitric acid and diluting to 1 liter. This solution was analyzed by the lead acid method and found to be 0.1981  $\pm$  0.0002M (average of four determinations).

#### PROCEDURE

To 8 grams of turnings in a 125-ml. Pyrex beaker add slowly 25 ml. of concentrated hydrochloric acid. After the first violent reaction has subsided, add cautiously a few milliliters of concentrated nitric acid, and warm to effect solution. When solution is complete, add sufficient nitric acid to make the volume added nitric acid 5 ml. Evaporate on a hot sand bath until salts begin to crystallize out, and the mixture boils like thick sirup. If desired, the evaporation may be hastened somewhat by careful heating on a wire gauze. Allow the mixture to cool for a short time, or until solid. Wash down with distilled water until about 10 ml. of water have been added.

Add 7 ml. of concentrated hydrochloric acid and heat on a sand bath until any hydrolyzed aluminum is redissolved. This requires 10 to 15 minutes. Transfer to a 50-ml. volumetric flask with the minimum of distilled water. Add 2.5 ml. of 0.2 per cent gelatin solution and 0.1 ml. of 2N hydroxylamine hydrochloride. Shake and heat until the solution becomes colorless. If necessary, add a second portion of hydroxylamine hydrochloride. (Occasionally a solution may remain colored in spite of a large excess of hydroxylamine. If the excess corresponds to 100 times the amount of iron present, this color can usually be ignored.) Dilute with freshly boiled distilled water, cool, and dilute to volume. Bubble with nitrogen in the electrolysis cell for 15 to 20 minutes to remove dissolved oxygen, and electrolyze from  $-0.04$  volt to the discharge potential of the supporting electrolyte (approximately  $-0.8$  volt) using a bridge potential. The sensitivity should be adjusted to give the largest possible steps in the curves.

The ferric iron is readily reduced to the ferrous state by treating the warm hydrochloric acid solution with hydroxylamine. In this state, the iron will not interfere with the determination of the copper, lead, and cadmium. Strubl (10) also made use of this reagent in the analysis of zinc blende which was high in iron.

The time required for a determination of copper, lead, and cadmium in a zinc-base alloy using the above procedure is about 3 hours. However, a large number of samples may be run at the same time, as only 10 minutes are required for a polarogram after the sample is prepared.

If copper is present in the alloy in excess of 0.1 per cent, it is advisable to remove the copper by electrodeposition from the acid solution as follows:

To 8 grams of zinc in a tall-form 250-ml. beaker, add 50 ml. of water and then add 23 ml. of nitric acid in small portions. When all the acid has been added, boil to complete solution, dilute to 100 ml. (or sufficient volume to cover the electrodes), and electrolyze at 4 amperes and 3 to 4 volts for 1 hour, with a rotating gauze anode and a gauze cathode. At the end of this time, wash down the cover glass and beaker and continue for another



minutes. Carefully remove the electrodes, while washing with a heavy stream of water. Under no circumstances should the circuit be broken before the electrode is completely free of acid. Rinse the electrode several times in 95 per cent alcohol, shake free of excess alcohol, and dry by revolving rapidly over a Bunsen flame after igniting the film of alcohol. Weigh as pure copper. Replace the anode, which may have lead oxide deposited, in the electrolyte and heat the whole to boiling. Then wash the electrode and remove it and boil the solution down to incipient crystallization. Add hydrochloric acid and carry out the procedure as for zinc which is low in copper. Care must be taken to remove all excess nitric acid. To do this, an extra evaporation with 10 ml. of hydrochloric acid is recommended before addition of the 7 ml. of hydrochloric acid and 10 ml. of water to redissolve the hydrolyzed aluminum.

This modification increases the total time required for an analysis, but when a large number of samples are to be run, this increase is considerably lessened. The results obtained for the copper by this method are usually slightly high (0.03 per cent high for 3 per cent copper). No traces of cadmium or zinc were found in the deposit when the deposit had been redissolved and deposited, and the electrolyte examined polarographically.

Table I. Calibration Constants for Copper, Lead, and Cadmium

Element	Leeds & Northrup			Sargent		
	1	2	Average	1	2	Average
	%/micro-ampere	%/micro-ampere	%/micro-ampere	%/micro-ampere	%/micro-ampere	%/micro-ampere
Cu	0.01815	0.01864	0.0184	0.02040	0.02078	0.0206
Pb	0.02905	0.03009	0.0296	0.03334	0.03413	0.0338
Cd	0.01869	0.01873	0.0187	0.01875	0.01888	0.0188

## RESULTS AND DISCUSSION

The capillary was calibrated by making additions of copper, lead, and cadmium from the diluted stock solutions. The calibration was carried out over a concentration range of  $10^{-6}M$  to  $3 \times 10^{-4}M$  on both the Leeds & Northrup and the Sargent polarographs. The step heights were obtained by difference from the residual current of the zinc used as a supporting electrolyte, and those produced by the zinc plus added ions. In the lower concentration range ( $1 \times 10^{-6}$  to  $5 \times 10^{-6}M$ ), this residual current amounted to from ten to twenty times the increase in current due to the added ions, and, accordingly a large error was introduced. This error was more significant when using the Leeds & Northrup instrument than when employing the Sargent apparatus. The fact that the latter has over twice the maximum sensitivity of the former would account in part for this difference in the results.

The calibration constants for two successive calibrations using two different zinc samples as a supporting electrolyte are given in Table I.

The factors were obtained in terms of per cent per microampere for each metal for the sake of convenience in calculating the values from the step heights. The basis for the calculation is an 8-gram sample in 50 ml. of solution.

In order to determine the precision of the method, and to discover the greatest source of error in the polarographic procedure, five series of determinations were carried out, using the Sargent instrument (Table II).

1. Precision of repeated determinations on the same cell.
2. Precision of repeated determinations on different aliquots of the same solution.
3. Precision of repeated determinations on different samples of the same alloy.
- 4 and 5. Precision of repeated measurements of the same curve by different individuals and by the same individual.

These results indicate that the mean deviation of measurement in all cases is approximately one half of the total mean deviation of the procedure. The deviation is not significant, however, in that it barely affects the fourth place of decimals. The over-all

Table II. Precision of Polarographic Procedure

Series <sup>a</sup>	Copper		Lead		Cadmium	
	Average %	Deviation <sup>b</sup> %	Average %	Deviation <sup>b</sup> %	Average %	Deviation <sup>b</sup> %
1	0.0018 <sub>1</sub>	0.00006	0.0057 <sub>9</sub>	0.00008	0.0005 <sub>8</sub>	0.00004
2	0.0018 <sub>1</sub>	0.00003	0.0058 <sub>7</sub>	0.00003	0.0006 <sub>2</sub>	0.00005
3	0.0018 <sub>5</sub>	0.00011	0.0056 <sub>6</sub>	0.00008	0.0006 <sub>2</sub>	0.00005
4	0.0010 <sub>4</sub>	0.00003	0.0017 <sub>1</sub>	0.00004	0.0009 <sub>5</sub>	0.00002
5	0.0010 <sub>2</sub>	0.00003	0.0016 <sub>7</sub>	0.00005	0.0009 <sub>3</sub>	0.00003

<sup>a</sup> Each of series 1, 2, and 3, is result of seven determinations. In series 3, each determination was made in duplicate. Series 4 is result of duplicate measurements by nine different individuals. Series 5 is result of ten measurements of same curve.

<sup>b</sup> Average deviation from arithmetical mean.

precision would indicate that it is possible to determine copper, lead, and cadmium to within  $1 \times 10^{-4}$  per cent for the range of concentrations encountered in high-purity alloys of the Mazak type.

At the time this investigation was carried out, it was impossible to procure a National Bureau of Standards zinc die-casting alloy of the type desired (Mazak 3). In lieu of this, an analysis was made on an alloy high in copper. Analyses are also presented for several standard zinc spelters (Table III).

It has been found, as a result of the analysis of a large number of commercially analyzed zinc samples, that the polarographic results are usually high. No explanation is available for this phenomenon. The calibrations have been checked and rechecked repeatedly in the authors' laboratory by different methods. There is a possibility that because of the small amount of handling, the polarographic results represent a closer approximation to the true value than analyses which are the result of many successive manipulations. The degree of precision of the polarographic procedure is high, as illustrated, and the deviations from other analyses do not show signs of a constant error. This is evidenced by examination of the above results for the Bureau of Standards samples.

Table III. Accuracy of Method

	(Sargent instrument)		
	Copper %	Lead %	Cadmium %
National Bureau of Standards. Sample 94			
Experimental	2.83 <sup>a</sup>	0.0318	0.0025
Precision	$\pm 0.01$	$\pm 0.0002$	$\pm 0.0001$
Certificate value	2.82	0.031	0.004
National Bureau of Standards. Sample 109			
Experimental	0.0007	0.0025	0.0019
Precision	$\pm 0.0001$	$\pm 0.0001$	$\pm 0.0001$
Certificate value	0.0005	0.0020	0.0018
National Bureau of Standards. Sample 108			
Experimental	0.0004	0.0505	0.0960
Precision	$\pm 0.0001$	$\pm 0.0002$	$\pm 0.0007$
Certificate value	0.0004	0.047	0.092

<sup>a</sup> By electrodeposition.

## INTERFERING ELEMENTS

There are some nineteen elements which may be found in zinc, either as impurities, or as alloying elements: Cu, Pb, Cd, Ni, Co, Mn, Ag, As, Hg, Tl, Bi, Sb, Al, Fe, Ge, Ga, In, Mg, and Sn. ("Interference" is intended to describe the preliminary or almost coincidental discharge of some undesired ion which either results in a masking of the step desired or makes impossible the recording of the desired ion at maximum sensitivity.)

Experiments with 0.05 per cent each of nickel, cobalt, manganese, silver, arsenic, mercury, and indium show that there is no detectable effect on the steps for copper, lead, and cadmium within  $\pm 0.0001$  per cent. Magnesium was tried up to 0.5 per cent and no interference was detected. This is to be expected, since the discharge potential of magnesium is well above that of



zinc. Copper up to 0.1 per cent has been determined polarographically without reducing the sample size while retaining a high degree of precision for lead and cadmium. Results of the electrodeposition of copper in conjunction with the polarographic determination of lead and cadmium indicate that there is no loss of lead and cadmium during this operation and that less than 0.01 per cent of the copper remains after deposition. Bismuth was found to give a step which precedes that of copper and for this reason interferes with the ensuing determination of copper, lead, and cadmium when present in excessive amounts. It is not close enough, however, to mask the copper step. The factor for bismuth was found to be approximately 0.024 per cent per A. for an 8-gram zinc sample in 50 ml. Antimony at 0.05 per cent gives a poorly defined step which interferes with the steps for copper, lead, and cadmium; however, at 0.01 per cent and less, no interference was found. Thus antimony concentrations of 0.01 per cent can be tolerated. The nature of the interference would seem to indicate a small diffusion coefficient for  $\text{Sb}^{++++}$  in this particular medium. Thallium gives a well-defined wave which comes between lead and cadmium in 2.5 zinc chloride. For trace amounts of copper, lead, and cadmium, only 0.002 per cent of thallium can be present. This corresponds to the findings of Seith and Esche (9) with regard to the limit of detection of thallium in zinc. Germanium was not tested for interference, because of the extreme volatility of its chloride. Gallium was not tested because of the difficulty in obtaining a salt of this metal. No interference is to be expected from gallium because of its high discharge potential.

Stannic tin when present in amounts greater than 0.0015 per cent will give a measurable increase in the step height for lead. Occasionally, amounts greater than this may be tolerated due to volatilization of stannic chloride, but such amounts of tin are not volatilized appreciably by this particular procedure. Kalovsek (6) indicates that the electroreduction of stannic tin is not reversible except in hydrochloric acid solutions of high concentration (above 0.1 N), where, however, the reduction process appears inhibited. This would explain why such a large amount of stannic tin would cause no interference. These results confirm those of Seith and Esche for the limit of detection of tin in zinc. Aluminum up to 6 per cent has been found to be without detectable effect on the height of the steps for copper, lead, and cadmium. Higher concentrations of aluminum might have an effect only in so far as they affected the concentration of the supporting electrolyte. Iron, after reduction with hydroxylamine hydrochloride is without significant effect in the determination of copper, lead, and cadmium at 2.5 per cent. It is necessary, of course, to adjust the amount of hydroxylamine hydrochloride used in accordance with the iron content for satisfactory results.

A study of the polarographic determination of trace quantities of tin, aluminum, and magnesium in high-purity zinc alloys is in progress.

#### CONCLUSION

Trace amounts of copper, lead, and cadmium can be rapidly determined by the polarographic method in high-purity zinc and zinc die-casting alloys with a high degree of precision. Such a method should prove of value in industrial laboratories where time is at a premium.

In an effort to make possible a complete polarographic analysis of high-purity zinc and zinc die-casting alloys, procedures are being developed for the determination of other elements present.

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## Determination of Pectin in Biological Materials Modification of Pentose-Furfural Method

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Pectin is converted to a pentose which produces the furfural with which this method is concerned. Data are presented to show the normal levels of furfural-yielding substances in various organs and fluids from rabbits. The analytical procedures described make it possible to recover 95 to 100 per cent of pectin which has been added to animal tissues and fluids.

**P**RESENT wartime conditions, which have increased interest in pectin sols for intravenous use in treating shock, have made it necessary to devise a semimicromethod for the determination of pectin in biological materials.

Pectinum N. F. VII which is suitable for intravenous use is essentially a pure polygalacturonic acid ester. When such a pectin is refluxed at elevated temperatures with 12.5 per cent hydrochloric acid the polygalacturonic anhydride units are decarboxylated, producing a mole of carbon dioxide for each carboxyl, and forming furfural from the newly formed pentose. Accurate quantitative procedures based upon the determination of the

carbon dioxide evolved and upon the furfural produced have been developed during the forty years which have elapsed since the general reactions were first described by Tollens (5).

The methods which have been developed for quantitative estimations of furfural are sensitive to extremely small amounts and adaptable to colorimetric procedures. The carbon dioxide methods are useful only when relatively large amounts of material are available; hence for purposes of determining pectin in biological systems the furfural scheme is preferred.

Furfural methods and their applications to pectin analysis were discussed by Browne and Zerban in 1941 (2). Youngburg (7) in 1927 described a particular adaptation of furfural estimation useful for biological materials. Bryant, Palmer, and Joseph (4) used a modification of Youngburg's method in these laboratories early in 1941 for an examination of the liver and other organs of the rabbit. The Youngburg scheme involved steam distillation from 85 per cent phosphoric acid and colorimetric determination of furfural in the distillate by the furfural-aniline acetate reaction. Details for the use of the Youngburg method



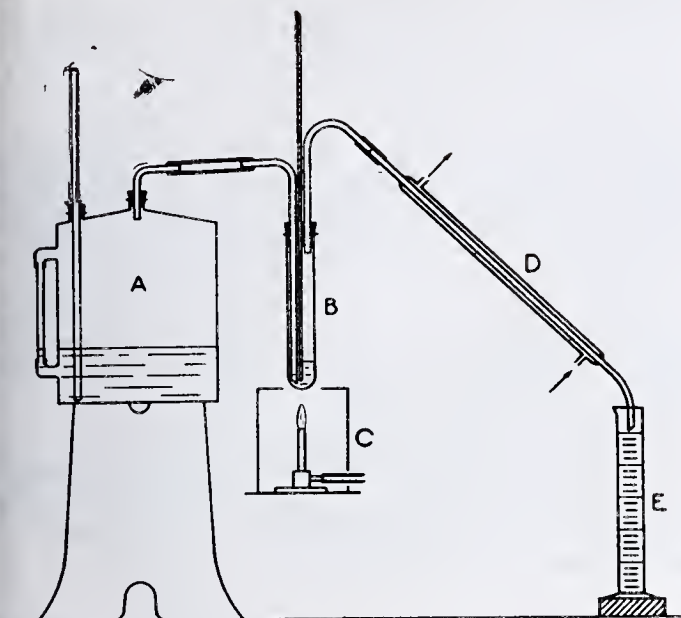


Figure 1. Distillation Apparatus

particularly the step involving trichloroacetic acid treatment of samples, were given by Andersch and Gibson (1).

Modifications of the Youngburg method previously published failed to allow reasonable recovery of pectin which had been added to urine, and also failed to provide samples from blood and tissue materials which could be distilled without excessive foaming. The method described here provides for an alcohol treatment of urine samples which permits complete recovery of pectin added to urine. It substitutes sodium tungstate for trichloroacetic acid and centrifuging for filtration, in preparing other samples, and continues with the regular Youngburg distillation. Foaming is eliminated and recoveries from control samples are excellent. The technique for the determination of furfural in the distillate by photoelectric colorimeter is described. The procedures given below are the result of several hundred analyses of rabbit blood, urine, and organs, and of many mixtures of pectin with these animal materials.

#### APPARATUS

The distillation apparatus shown in Figure 1 is composed of steam generator, A, made from a large ether can with burner underneath regulated by screw clamp for control of steam flow into B, the distillation unit, a Pyrex 25 × 150 mm. test tube, with thermometer covering the range 0° to 200° C.; C, tin cylinder shield for microburner; and D, a water-cooled condenser, 250-mm. jacket, with inside tube preferably 6 to 7 mm. in diameter and turned down at end for delivery into E, a 50-ml. graduated cylinder. An ordinary steam generator made from glass may be used instead of the metal one described.

A number of the Pyrex 25 × 150 mm. test tubes should be available because urine samples are prepared in them and then may be stored until time is available for distillation. It is convenient to have 2.0-, 3.0-, 4.0-, and 5.0-ml. volumetric pipets, as well as Mohr pipets, 1.0-ml. size graduated in 0.10 divisions. A centrifuge at least as large as the International No. 1 and several 10-ml. centrifuge tubes are required. A photoelectric colorimeter is recommended. The Fisher Electrophotometer with a blue filter (No. 425B) and the Klett-Summerson photoelectric colorimeter with a green filter (No. 54) have been used successfully with this method.

#### REAGENTS

Freshly prepared furfural, vacuum-distilled at a pressure of 0 to 30 mm.

Standard furfural stock solution prepared from the freshly distilled furfural; 1.000 gram is diluted to 500 ml. with distilled water. This stock solution may be kept for several weeks in a refrigerator.

Standard dilute solution of furfural for calibration of photoelectric colorimeter or as a comparison solution with the Duboscq-type colorimeter. This solution is made by diluting 5.0 ml. of the stock to 1000 ml. with distilled water. This dilute

standard (1.00 ml. equivalent to 0.01 mg. of furfural) should be made fresh the day of use.

Freshly distilled aniline, free from furfural. Test by adding 0.25 ml. to 2.0 ml. of glacial acetic acid. If a pink color develops within one minute, furfural is present.

Glacial acetic acid, 85 per cent orthophosphoric acid, 10 per cent by weight solution of sodium tungstate ( $\text{Na}_2\text{WO}_4 \cdot 2\text{H}_2\text{O}$ ), 0.5N sulfuric acid, and isopropyl or ethyl alcohol, at least 95 per cent.

#### PREPARATION OF SAMPLES

**PECTIN SOLS FOR CHECK ON METHOD.** The utility of this method in determining the fate of injected pectin depends upon having an accurate figure for the furfural equivalent per milliliter of the pectin sol being used in the animal experiments. Each lot of Exchange Pectinum N. F. VII will have a definite value for the furfural equivalent, usually varying from 190 to 215 mg. of furfural per gram of pectin. These furfural values, obtained by the method described below, have been checked by using the standard gravimetric phloroglucin method (6). The values by the two methods agree almost perfectly.

Animal work with pectin usually involves 1.0 to 2.0 per cent sols which have been sterilized by autoclaving and filtered to sparkling brilliance. Standardization analyses on such sols should be run on samples prepared as follows: 5.0 ml. of 1.0 to 2.0 per cent pectin sol, diluted to 200.0 ml. with distilled water; 2.0 ml. of this diluted sol should be used for each analysis.

**URINE.** Normal urine from rabbits usually contains so little furfural-yielding material that 4.0-ml. samples are required for an analysis. When pectin has been injected into an animal, it is necessary to use only a 2.0-ml. sample of urine to get good results, while pectin is being excreted.

Pipet duplicate samples of urine into 25 × 150 ml. Pyrex test tubes and add 10 volumes of at least 95 per cent alcohol. After standing for an hour or two centrifuge the tubes and pour off the supernatant liquor. Place the tubes containing the residue in a drying oven at 100° C. and dry for about 30 minutes, or until no odor of alcohol can be detected. These tubes containing the dried residue may then be covered and stored until ready for analysis.

**URINE AND ADDED PECTIN, AS CONTROL ON METHOD.** Prepare a check solution by diluting 5.0 ml. of a 1.0 to 2.0 per cent pectin solution with urine up to 200.0 ml. in a volumetric flask, and treat 2.0-ml. samples with alcohol as described above.

**BLOOD.** Prepare a Folin-Wu blood filtrate by the following method: Weigh 2.0 ml. of oxalated blood (10 mg. of potassium oxalate per 5 ml. of blood) accurately in a 30-ml. centrifuge tube. Add to this by pipet 13.0 ml. of distilled water, 2.0 ml. of 10 per cent sodium tungstate solution, and 3.0 ml. of 0.5N sulfuric acid. Mix the contents of the tubes well, allow the samples to stand 15 minutes, then centrifuge at about 1000 r.p.m. for 30 minutes. Remove the clear centrifugate and save for analyses, using 2.0 ml. for each sample, as described below.

**ANIMAL TISSUES.** Analyses may be made upon the organs immediately after they have been removed from the animal and weighed, or the material may be frozen with dry ice and stored for later use. In either case it is necessary to macerate the organ (or a portion of it in cases of large organs) in a mortar. Transfer as much as possible from the mortar (not to exceed 10 grams of the larger organs), into a tared beaker and then add about 10 times as much distilled water as the weight of the organ used. Stir this weighed mixture thoroughly and transfer to a mixer such as the Waring Blendor where it is reduced to a homogeneous slurry. Pipet a volume of this slurry equivalent to about 0.3 to 0.5 gram of the original organ into a 30-ml. centrifuge tube, and weigh. Add distilled water to bring the volume to 15.0 ml., then add 2.0 ml. of 10 per cent sodium tungstate solution and 3.0 ml. of 0.5N sulfuric acid, mixing the contents of the tube by careful swirling after the addition of each reagent. Mix the contents of the tubes well and allow the samples to stand 15 minutes. Then centrifuge at about 1000 r.p.m. for 30 minutes. Remove the clear centrifugate and save for analyses, using 2.0 ml. for each distillation.

#### METHOD

The sample for analysis should be in a 25 × 150 mm. Pyrex test tube, prepared as discussed under "Preparation of Samples".

Add to the sample in the test tube 5.0 ml. of 85 per cent phosphoric acid and connect with the condenser and the steam generator. Light the microburner and raise the temperature rapidly to 170° to 175° C. The temperature should be maintained at this point and never allowed to go higher during the 20- to 30-minute distillation period.

Collect 40 ml. of distillate in the 50-ml. graduated cylinder used as a receiver, and an additional 10 ml. of distillate in a test tube



previously marked at the 10-ml. level. Test this last distillate for the presence of furfural as follows: Mix 2.0 ml. of the distillate, 0.25 ml. of aniline, and 2.0 ml. of glacial acetic acid in a test tube. If no color develops in one minute, stop the distillation and discard the final 10 ml. of distillate. If color does appear in the test sample, distill an additional 10 ml. and test a portion for furfural as above described. When analyzing blood or urine of untreated animals, 30 ml. of distillate are usually sufficient to contain all the furfural.

Table I. Recovery of Pectin Added to Rabbit Urine

Material Analyzed	Furfural Found	
	Youngburg method	Present method
	Mg.	Mg.
2.0 ml. of 1.75% pectin sol B-9221	7.08	7.08
48.0 ml. of urine + 2.0 ml. of pectin sol B-9221	11.46	9.22
48.0 ml. of urine + 2.0 ml. of distilled water	7.33	2.08
Difference, due to added pectin	4.13	7.14
Recovery of added pectin, %	58.3	100.9

Table II. Furfural Equivalent of 1.00 Gram of Pectinum N. F. VII

(Sample 444-H-3. Analyses made on water solution containing 750 mg. of pectin per liter)

Operator	Furfural Found	
	Mg./gram 444-H-3	
E.F.B.	201	
G.H.P.	198	
G.H.P.	205	
G.H.P.	200	
Average value used	201	

Combine and mix 20.0 ml. of the original 40-ml. distillate and 5.0 ml. of each 10-ml. distillate showing a positive test for furfural. Place 5.0 ml. of this mixture (or 5.0 ml. of the original 40-ml. distillate when the furfural test in the next 10 ml. was negative) in a tube and mix with 0.5 ml. of aniline and 4.5 ml. of glacial acetic acid, carefully measured with a pipet.

Set this mixture aside in the dark at 20° to 25° C. for exactly 15 minutes, at which time the color intensity is determined with a photoelectric colorimeter. This 15-minute period has been determined experimentally as giving the most exact value. The colorimeter should be calibrated by using the standard dilute furfural solution described above, spacing samples over the range from 0.001 to 0.050 mg. of furfural per 10 ml. of solution containing 0.5 ml. of aniline, 4.5 ml. of glacial acetic acid, and the diluted standard furfural solution.

When using the Duboscq type of instrument a standard for colorimetric comparison must be prepared for each sample. This is done by mixing 2.0 ml. of the dilute standard furfural solution made that day, with 3.0 ml. of distilled water, 0.5 ml. of aniline, and 4.5 ml. of glacial acetic acid. The tube containing this mixture and also the one with the unknown are set aside in the dark for exactly 15 minutes, for color development. The unknown and the standard should have about the same color intensity. If they do not, the solutions should be remade, reducing the volume of either the unknown or standard, adjusting the total volumes with distilled water to keep them the same as outlined above.

#### DISCUSSION

An illustration of the accuracy of the present method compared with that of the original Youngburg scheme, in the case of rabbit urine, is given by Table I.

It was found that urea added to pectin sols decreased the recovery of pectin by the Youngburg procedure. Many analyses, as illustrated by Table I, show that even though the alcohol precipitation scheme does remove some of the naturally occurring aldehyde-producing substances of urine, it also removes the urea and thereby allows complete recovery of the added pectin. The increase in furfural due to added pectin in urine (human as well as rabbit), using the alcohol precipitation method, has always been found equivalent to the furfural obtained from the pectin when analyzed alone.

When this method is used to follow pectin excretion from animals to which pectin sol transfusions have been given, it is necessary to know the furfural equivalent for the pectin sol used and the normal furfural values for the organs or fluids being examined.

It is desirable to know the actual pectin concentration of the pectin sol used or else the furfural equivalent per gram of the pectin used to make the sol. The typical examples of these data shown in Tables II and III illustrate the ranges for these values as well as show the precision and accuracy of the method.

The data in Table III show that 100 ml. of solution B-9221 would be equivalent to 355 mg. of furfural; hence the solution must be 355/201 or 1.76 per cent pectin. Pectin sol B-9221, when analyzed by a modified Lefevre-Tollens method (described by Bryant and Joseph, 3), showed 0.2315 gram of galacturonic acid per 15.0-ml. sample. Pectin 444-H-3 had previously been found by the same method to contain 87.6 per cent of galacturonic acid; hence the 15.0-ml. sample of B-9221 contained 100 (0.2315)/15.0 × 0.876 or 1.76 per cent of pectin.

The extent to which furfural-producing substances occur in rabbit organs is indicated in Table IV, along with analytical values showing how the present method eliminates interference from the various animal materials and permits practically complete recovery of any added pectin.

Table III. Furfural Equivalent of 1.00 Ml. of Autoclaved 1.75 Per Cent Isotonic Pectin Sol

(Sol B-9221 made from Pectin 444-H-3. Analyses made on dilution of 5 ml. of B-9221 to 200 ml. total)

Operator	Date	Furfural Mg./ml. B-9221
G.H.J.	Sept. 11, 1942	3.59
G.H.P.	Sept. 14, 1942	3.46
E.F.B.	Oct. 5, 1942	3.54
G.H.J.	Oct. 7, 1942	3.57
E.F.B.	May 27, 1943	3.58
G.H.J.	July 21, 1943	3.54
E.F.B.	July 21, 1943	3.56
Average value		3.55

Table IV. Furfural Obtained from Rabbit Tissues and Recovery of Pectin Added to These Tissues

(1.00 ml. of pectin sol B-9221 added is equivalent to 3.55 mg. of furfural (Table III))

Material	Sample Weight Grams	Furfural Obtained		Furfural Due to Added Pectin Mg.	Recovery of Added Pectin %
		Original tissue sample Mg.	Tissue plus 1.0 ml. of pectin sol B-9221 Mg.		
Blood	1.903	0.23			
	1.539		3.80	3.61	101.7
Bile	0.320	0.29			
	0.320		3.71	3.42	96.3
Bone	0.500	0.10			
	0.500		3.62	3.52	99.2
Heart	0.330	0.14			
	0.330		3.62	3.48	98.0
Kidney	0.535	0.22			
	0.535		3.74	3.52	99.2
Liver <sup>a</sup>	0.492	1.95			
	0.496		5.60	3.65	102.8
Spleen	0.320	0.14			
	0.320		3.62	3.48	98.0

<sup>a</sup> Better results are obtained when sample weight of liver is about 0.2-0.3 gram.

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### Studies on the Chemistry of the Fatty Acids Absorption Spectra Analysis of Conjugation in Fatty Acid

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A method of determining the amount of two, three, and four double bond conjugation in the presence of nonconjugated unsaturated fatty acids has been used in the analysis of samples of linoleic acid. The results indicate that the recrystallization method of purification gives a product more nearly free from conjugation than is obtained by debromination procedures. The conjugation rearrangement is probably caused by the zinc bromide formed during the debromination, and the  $\beta$  form of the acid contains a comparatively high percentage of conjugated material.

SAMPLES of linoleic acid of high purity have recently been prepared by both the classical method of debromination of the tetrabromide (5) and the fractional crystallization of the free acid from corn oil (4, 6). Since these samples were known to contain small amounts of conjugated unsaturated fatty acids, an absorption spectra study was made to obtain a more accurate estimate of the amount of these impurities. This knowledge, in turn, should help to determine the method of preparation that will lead to the purest product.

The purpose of this investigation has been not only to present the data observed on linoleic acid, but also to indicate a more accurate method of analyzing the absorption spectra of the conjugated unsaturated fatty acids. Spectroscopic methods have been previously used to indicate the amount of two, three, and four double bond conjugation in samples of fatty acids. By one of these procedures (12, 15), the amount of conjugation was indicated by comparing the values found for the extinction of a 1% solution in a 1-cm. cell ( $E_{1\text{ cm.}}^{1\%}$ ) at each of the three frequencies characterized by the most intense band in unsaturated acid samples containing two, three, and four conjugated ethylene groups. In another procedure (1), the highest value of

$E_{1\text{ cm.}}^{1\%}$  at each of the three characteristic frequencies was set at 100 and by direct proportion other values were converted to the same scale, thus giving per cent. In both methods, however, the assumption has been made that all the absorption in the portion of the spectrum characteristic of a given combination of ethylene linkages was due entirely to the arrangement of double bonds in the mixture being analyzed. Since the pure conjugated acids have absorption bands that overlap, some error is introduced by the above assumption, and hence the method presented in this paper has been prepared to correct for this error.

#### EXPERIMENTAL

All the samples of linoleic acid used were prepared by Frankel and Brown (5, 6), with the exception of the first which was prepared by Matthews, Brode, and Brown (11). The analytical constants of the acids are summarized in Table I. The form of the acid, indicated as  $\alpha$  or  $\beta$ , does not have a structural significance, but indicates whether the tetrabromide used to form the acid was insoluble ( $\alpha$ ), or soluble ( $\beta$ ), in petroleum ether. In all cases the soluble tetrabromide used in preparing the  $\beta$ -acid was prepared from an  $\alpha$  form comparable in purity to that of sample 3.

To make the ultraviolet absorption measurements, a Bausch & Lomb medium quartz spectrograph, with a modified Hilger spectrophotometer attached, was used in conjunction with a hydrogen arc. The detailed procedure followed was similar to that already reported (2).

Examples of absorption spectra curves are shown in the accompanying illustrations. Figure 1 shows the curve obtained for the first sample and indicates no resolution of the fine structure. Although sample 4 (Figure 2) has a smaller percentage of total conjugation, the bands caused by the conjugation of three double bonds are clearly resolved. The reason for this is obvious

Table I. Analytical Constants of Linoleic Acid Samples

Sample	Form of Acid	Method of Preparation	Solvent Used in Debromination	M.P. ° C.	Iodine Value	Tetrabromide No.	Linoleic Acid from Iodine value %	Tetrabromide No. %	Isomeric Acid* %
1	$\alpha$	Debromination, 12 recrystallizations	Methyl alcohol (11)	-5.2 to -5.0	181.0	102.9	...	100	0.0
2	$\alpha$	Debromination <sup>b</sup>	...	-7.0	180.9	90.6	100	88	12
3	$\alpha$	Debromination	Methyl alcohol	-5.4	180.8	100.6	100	98	2
4	$\alpha$	Crystallization	(4, 6)	-20.2	167.8	52.4	85	51	34
5	$\beta$	Debromination	Methyl alcohol	-2.0	173.5	21.3	91	21	70
6	$\beta$	Debromination	Pyridine	-22.2	166.3	43.6	83	42	41
7	$\beta$	Debromination	Isopropyl ether	-21.8	169.0	44.6	87	43	44
8	$\beta$	Debromination	Diethyl ether	1.0	89.8	...	94	46	48
9	$\beta$	Debromination	Acetic acid	-22.2	174.1	47.7	...	...	...
10	$\beta$	Debromination	Dioxane	...	...	...	...	...	...

\* Based on difference between % of linoleic found from iodine value and tetrabromide number.

<sup>b</sup> Mixture of several specimens of  $\alpha$ -acid, prepared by debromination in various solvents (5).

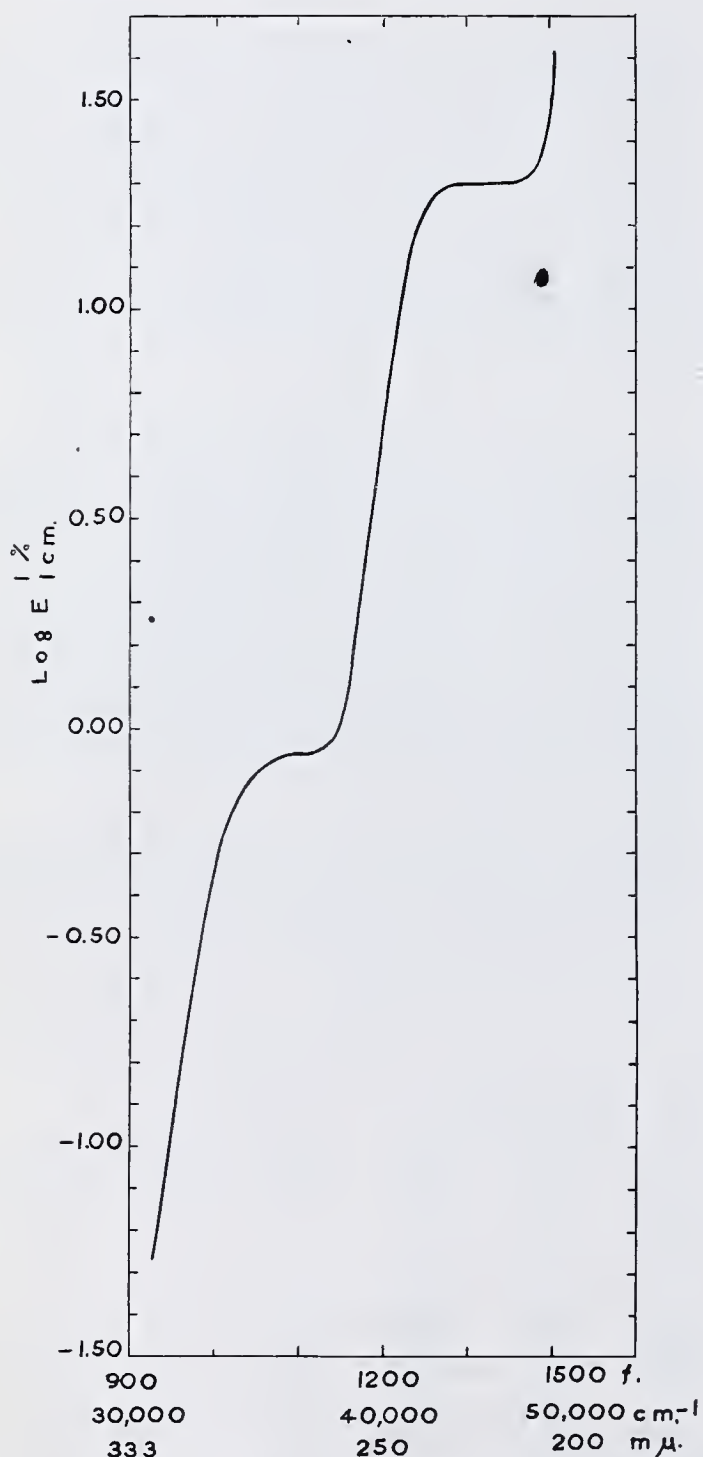


Table II.  $E_{1\text{ cm.}}^{1\%}$  Observed for Linoleic Acid Samples

Frequency ( $\nu$ ) Wave Number ( $\text{cm.}^{-1}$ ) Wave Length ( $\text{m}\mu$ )	Number of Conjugated Ethenylene Linkages						
	4 938 31,250 320	4 977 32,550 307	4 1028 34,250 292	3 1071 35,650 280	3 1110 37,000 270	3 1160 38,650 258	2 1290 43,000 233
Sample 1	0.0725 <sup>a</sup>	.....	.....	0.835 <sup>a</sup>	0.872 <sup>a</sup>	...	13.5 <sup>a</sup>
2	0.0617	0.135	0.316	0.780	1.01	...	12.7 <sup>a</sup>
3	0.960	1.32	.....	3.39	3.72	3.09	8.75 <sup>a</sup>
4	0.725	.....	0.355 <sup>a</sup>	2.19	2.48	2.16	3.72 <sup>a</sup>
5	0.417	.....	3.63 <sup>a</sup>	10.1	13.2	10.2	14.0
6	0.589	.....	.....	10.1	13.8	11.88	25.2
7	0.537	1.01 <sup>a</sup>	3.47 <sup>a</sup>	18.6	24.1	18.4	37.2
8	0.562	0.891 <sup>a</sup>	12.9 <sup>a</sup>	30.2	37.8	32.8	52.2
9	0.0725	0.145	0.479 <sup>a</sup>	1.29	1.66	...	39.8
10	0.612	1.00 <sup>a</sup>	6.17 <sup>a</sup>	21.8	25.4	22.0	40.7 <sup>a</sup>

<sup>a</sup> Infection.

when the intensities of the maxima caused by two conjugated double bonds are compared in the two graphs. Figure 3 is included to show the resolution of the bands produced by the

Figure 1. Absorption Spectra of Linoleic Acid  
Sample 1 in 95% ethyl alcohol

conjugation of four double bonds. Sample 2 gives a curve similar to that shown in Figure 2, while all the  $\beta$  forms of the acid are best illustrated by Figure 4. The data from the ultraviolet absorption spectra are summarized in Table II.

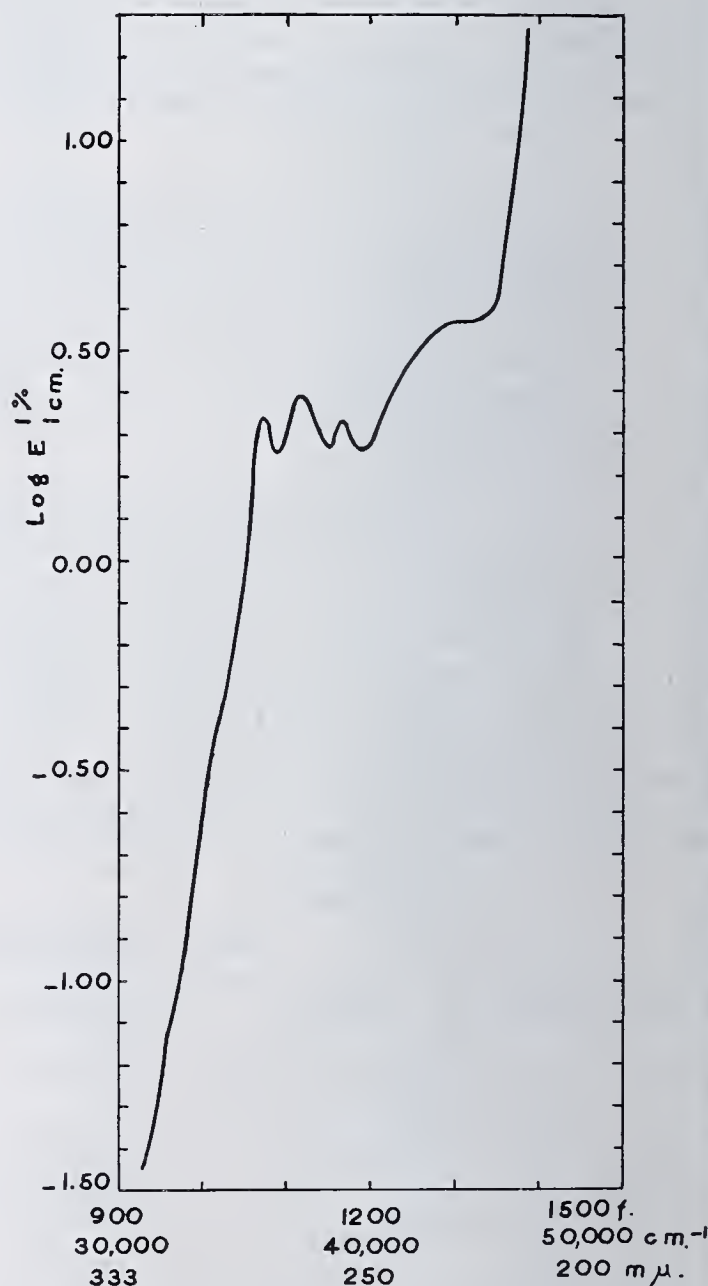
## METHOD OF ANALYSIS

Since  $[E_{1\text{ cm.}}^{1\%}]_{\nu}$  at any frequency,  $\nu$ , is directly proportional to the concentration, it is possible to calculate the percentage of a substance present in a sample by dividing the observed value of  $[E_{1\text{ cm.}}^{1\%}]_{\nu}$  by that of the pure substance, provided the absorption is caused entirely by one substance. If the absorption is due to more than one substance, the observed value of  $[E_{1\text{ cm.}}^{1\%}]_{\nu}$  will be equal

to the sum of the individual contributions of each of the components, or in a three-component system:

$$100 [E_{1\text{ cm.}}^{1\%}]_{\nu} = [xL]_{\nu} + [yM]_{\nu} + [zN]_{\nu}$$

where  $L$ ,  $M$ , and  $N$  are the values of  $[E_{1\text{ cm.}}^{1\%}]_{\nu}$  of each of the components in the pure state at frequency  $\nu$  and  $x$ ,  $y$ , and  $z$  rep-

Figure 2. Absorption Spectra of Crystallization Linoleic Acid,  
Sample 4



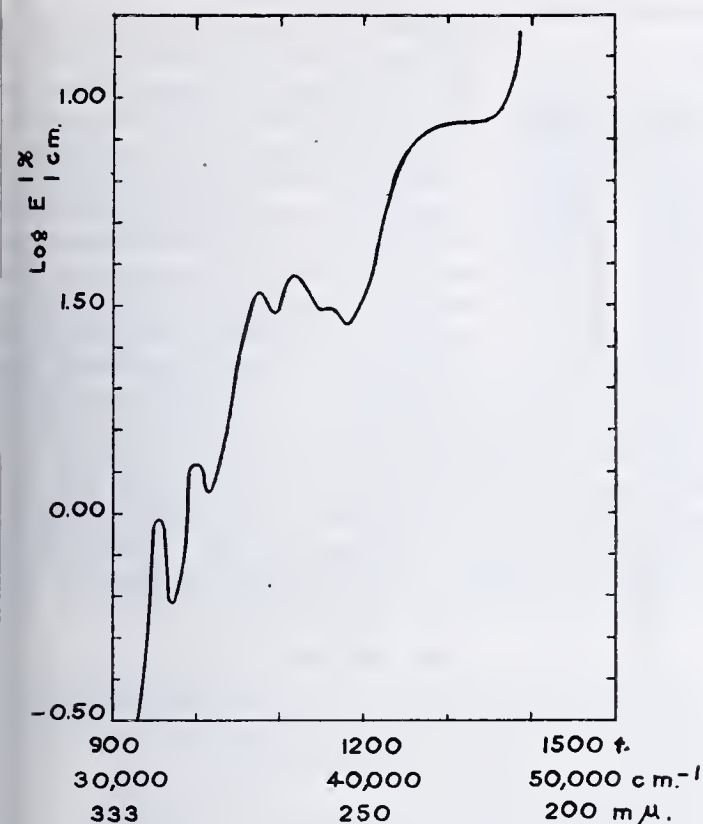


Figure 3. Absorption Spectra of Linoleic Acid, Sample 3

represent the per cent of each of the substances in the mixture. The values of  $L$ ,  $M$ , and  $N$  at any selected frequency can be obtained from the absorption spectra of the pure substances and  $[E_1^{1\%}]_{\nu}$ ,  $x$ ,  $y$ , and  $z$  remain to be determined. The extinction value,  $[E_1^{1\%}]_{\nu}$ , must be measured at three different frequencies in order to provide three equations, which can be solved simultaneously to determine the per cent of each of the three components.

In order to apply this method to the determination of the amount of two, three, and four conjugated double bonds in fatty acids, it is necessary that the absorption curves of the pure material containing each of these resonating systems be available. The data used for these standards are taken from the literature and summarized in Table III.

Table III.  $E_1^{1\%}$  Values of Standard Curves

No. of Conjugated Ethylene Linkages	Literature Reference	938 f. 31,250 cm. <sup>-1</sup> 320 mμ	1,071 f. 25,650 cm. <sup>-1</sup> 280 mμ	1,290 f. 43,000 cm. <sup>-1</sup> 233 mμ
2	(8, 10)	.....	0.1 <sup>a</sup>	1200
3	(3, 8)	1.0 <sup>a</sup>	1320	112
4	(9, 13, 14)	2000	700	60

<sup>a</sup> Exterpolated.

The simultaneous equations may be set up as follows:

$$1.0y + 2000z = 100A = 100E_1^{1\%} \text{ at } 938f.$$

$$0.1x + 1320y + 700z = 100B = 100E_1^{1\%} \text{ at } 1071f.$$

$$1200x + 112y + 60z = 100C = 100E_1^{1\%} \text{ at } 1290f.$$

$A$ ,  $B$ , and  $C$  represent  $E_1^{1\%}$  observed at the respective frequencies, and  $x$ ,  $y$ , and  $z$  represent the percentage of two, three, and four conjugated double bonds, respectively. On solution of the equations these results are obtained:

$$x = 0.0833C - 0.00706B + 0.00001A$$

$$y = 0.0758B - 0.0268A - 0.00000623C$$

$$z = 0.0500A - 0.0000379B - 0.000000031C$$

The coefficient of  $A$  in the equation for  $x$  and of  $C$  in the equation for  $y$  is small compared to the other coefficients in the equa-

tions. This is also true of the coefficients of  $B$  and  $C$  in the third equation. For instance, in the first of these equations the coefficient of  $A$  is only  $1/1000$  that of  $C$ , and since the value of  $A$  never exceeds that of  $C$  in any of the samples studied, the term containing  $A$  is negligible. In the same way, it can be shown that the last term in the second equation and the last two terms in the third contribute an insignificant amount to the values of  $y$  and  $z$ , respectively. When these terms are omitted the equations become:

$$x = 0.0833C - 0.00706B$$

$$y = 0.0758B - 0.0269A$$

$$z = 0.0500A$$

Having obtained these equations, the percentage of each type of conjugation is found by substituting in the appropriate equation and solving for  $x$ ,  $y$ , or  $z$ .

Several samples of linoleic acid have been analyzed in this manner and the results are compared with others, calculated as in the earlier method (1), in Table IV. Inasmuch as the percentage difference in some cases is small, it should be noted that it increases as the ratio of the higher to the lower types of conjugation increases and will become very large if, for instance, the amount of three and four double bond conjugation is equal.

#### DISCUSSION OF RESULTS

A comparison of Tables I and IV indicates some interesting results concerning the best method of purification of linoleic acid in order to keep the percentage of conjugation at a minimum. The fact that the lowest total conjugation is found in sample 4 is a strong recommendation for crystallization methods of purification (6). It is conceivable that this total might be further reduced by recrystallization, which should remove the three and four double bond portions.

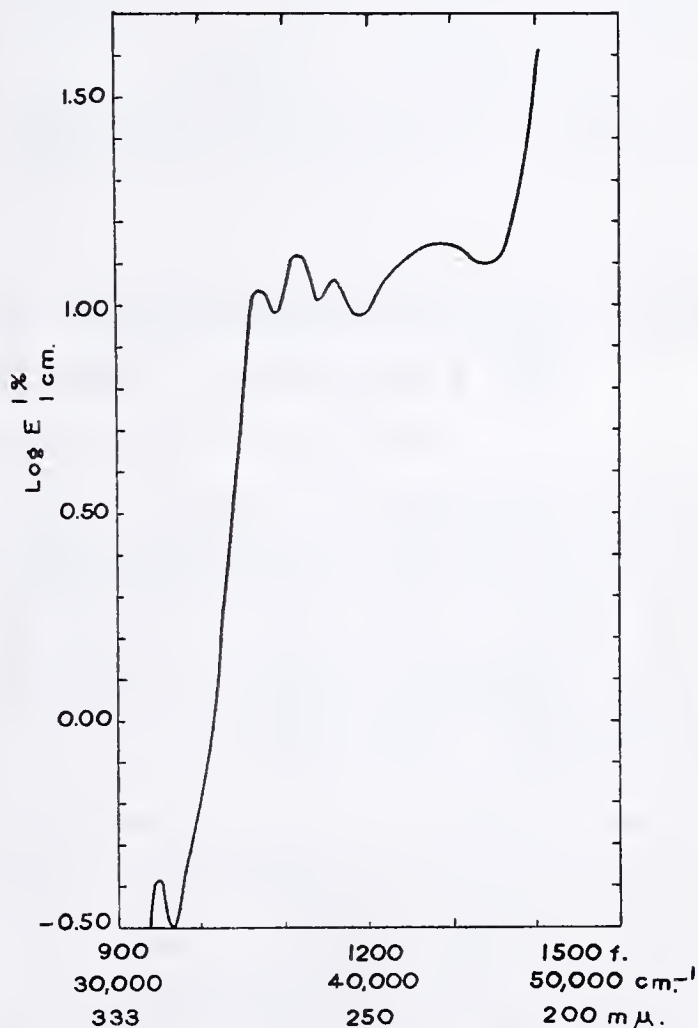


Figure 4. Absorption Spectra of Linoleic Acid, Sample 5



Table IV. Percentage Conjugation in Linoleic Acid Samples

Sample	Number of Conjugated Ethenylene Linkages							
	New or old method	Old method	New method	Difference, %	Old method	New method	Difference, %	Total conjugation, %
1	0.0034	0.063	0.061	313	1.1	1.1	0.0	1.2
2	0.0031	0.059	0.057	1.7	1.1	1.1	0.0	1.2
3	0.048	0.26	0.23	13	0.73	0.70	4.2	0.98
4	0.0034	0.17	0.17	0.0	1.31	0.29	6.6	0.46
5	0.021	0.76	0.75	1.3	1.2	1.1	9.1	1.9
6	0.030	0.77	0.75	2.7	2.1	2.0	5.0	2.8
7	0.027	1.41	1.41	0.0	3.1	3.0	3.3	4.4
8	0.028	2.30	2.30	0.0	4.4	4.1	7.5	6.4
9	0.0034	0.098	0.096	2.1	3.3	3.3	0.0	3.4
10	0.031	1.6	1.6	0.0	3.4	3.2	6.2	4.8

A comparison of the isomeric acid factor (right-hand column in Table II) with the total per cent conjugation of the same samples as shown in Table IV shows a reasonable agreement between these two data with regards to relative values of the  $\alpha$ - and  $\beta$ -acid types, the  $\alpha$  values being lower in all cases.

Debromination methods of isolation not only tend to conjugate the unsaturated bonds of linoleic acid but also produce considerable amounts of octadecadienoic acids which do not yield insoluble tetrabromides, as is indicated by samples 1 and 4 as compared with samples 5 to 10. The fact that sample 3 contains comparatively large amounts of the three and four conjugation is probably accidental, since none of the samples of the  $\beta$ -acid which have been through two debrominations have as high a percentage of four double bond conjugation. The origin of the conjugation cannot be accredited to alkaline saponification (1, 10, 15), inasmuch as the crystallization sample has also been through the same treatment during the preparation of the free acid from the oil. A recent publication of Henne and Turk (7), which reports the conjugation of diolefins with metallic halides, perhaps affords the best explanation of the cause of this conjugation, since zinc bromide is formed as one of the products during the debromination and is probably responsible for the shift in the position of the ethenylene linkage.

The samples of  $\beta$ -acids are the products of two debromination treatments and as such should have about twice as much conjugation as the  $\alpha$  forms. However, the data indicate that

in most cases the per cent conjugation is much more than twice that in the  $\alpha$  form and in one instance (sample 8) the per cent of conjugation of the  $\beta$  form is five times as great as that of the highest  $\alpha$  form. This result is not unexpected, inasmuch as the tetrabromide used to form the  $\beta$ -acid is a mixture of stereoisomers which yield, on debromination, geometrically isomeric acids (4, 11). It is possible, then, that one or more isomers more readily conjugated than the  $\alpha$ -acid are the cause of the high percentage of conjugation in the  $\beta$ -acids. The effect of

the solvent on the isomerization is indicated in the results, and permits the listing of solvents in the order in which they give increasing amounts of conjugation—methyl alcohol, pyridine, isopropyl ether, dioxane, and diethyl ether. Acetic acid is not listed, since it may react with zinc during the debromination and reduce some of the ethenylene linkages.

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## Electrolytic Determination of Copper in Steel and Cast Iron

### With a Supplementary Colorimetric Procedure for Certain Alloy Steels

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Copper is determined electrolytically in the presence of ferrous iron with the use of an Alundum thimble as a diaphragm. The sample is dissolved in dilute sulfuric acid and the copper oxidized by ferric sulfate or nitric acid. Satisfactory results have been obtained on carbon and low-alloy steels and cast irons by direct weighing of the cathode. Steels containing much molybdenum, tungsten, or chromium require that the plating be dissolved and the copper determined colorimetrically. The simplicity and the time required for a determination compare favorably with existing methods.

THE present A.S.T.M. (1) methods for the determination of copper in steel are the electrolytic or gravimetric and the thiosulfate-iodide volumetric. Lundell, Hoffman, and Bright (4) describe the thiocyanate-iodide volumetric and the colorimetric-ammonia methods for determining copper. All these methods require a number of time-consuming steps, and a more direct method of determining copper is desirable for routine work.

Frediani and Hale (2) report an electrolytic method in which the copper is plated out without separation of the iron. The

iron is present as the ferric phosphate complex and a temperature of 10° C. is required. Silverman, Goodman, and Walter (8) describe another electrolytic method in which copper is deposited from a solution containing all the iron in the trivalent state. No agitation is employed. A voltage of 6.0 is used and about 2 hours are required for complete deposition. Sand (7) determines copper in steel by means of an internal electrolysis method.

In the method reported here, the copper is plated from a solution containing approximately 5 grams of ferrous iron in about 45 minutes. A voltage of 2.1 to 2.2 is used with a minimum of special equipment.

## SPECIAL REAGENTS AND SOLUTIONS

**SULFURIC ACID, 4%.** Add 40 cc. of concentrated sulfuric acid to 960 cc. of water to make approximately 4% sulfuric acid.

**FERRIC SULFATE.** Place 100 grams of  $\text{Fe}_2(\text{SO}_4)_3 \cdot x\text{H}_2\text{O}$  in 1-liter beaker, add about 750 cc. of water and 50 cc. of concentrated sulfuric acid, and heat the mixture slowly with frequent stirring until the reagent has all dissolved. Cool and dilute to one liter.



**SODIUM THIOCYANATE SOLUTION.** Dissolve 5 grams of sodium thiocyanate in 100 cc. of water.

**SODIUM DIETHYLDITHIOCARBAMATE SOLUTION, 0.2%.** Dissolve 0.2 gram of the reagent in 100 cc. of water.

**DILUTE AMMONIUM HYDROXIDE SOLUTION.** Mix 200 cc. of concentrated ammonium hydroxide with 800 cc. of water.

#### PROCEDURE FOR CARBON STEELS AND CAST IRONS

Use a 5-gram sample for steels containing less than 0.5% copper and a correspondingly smaller weight for those higher in copper. Place the sample in a 200-cc. tall-form beaker, add 92 cc. of distilled water and 8 cc. of concentrated sulfuric acid, cover the beaker with a watch glass, and heat until the iron is dissolved. The insoluble residue remaining contains most of the copper in the sample. Wash down any residue on the sides of the beaker, heat the solution, and when it is boiling vigorously, add 5 cc. of the ferric sulfate solution. Continue the vigorous boiling for 5 minutes. This treatment oxidizes the undissolved copper in the residue to divalent copper. With a glass tube of small bore, transfer a small amount of the solution to a spot plate, and add a drop of the sodium thiocyanate solution to the test sample on the spot plate. If the resulting color is a bright cherry red, the oxidation is complete and the proper amount of excess ferric ion is present. If the resulting color is a pale pink, all the copper may not be oxidized. In this case, add 3 cc. more of the ferric sulfate solution, boil 5 minutes, and retest. For most steels, one addition of the ferric solution will be sufficient. Filter the solution, now containing all the copper as the cupric ion, through a Whatman No. 7 paper into a tall-form 200-cc. beaker, wash the residue 5 times with cold water, and discard it. For routine work, the residue need not be filtered off. Dilute the filtrate to 150 cc. with distilled water and cool to a temperature around 15° to 18° C. in running cold water. The sample is now ready for electrolysis.

The apparatus used for the deposition of copper is shown in Figure 1.

A represents the 200-cc. electrolytic beaker containing the dissolved sample. It is placed on top of the wooden block, L. The power source is a 6-volt, 150-ampere motor generator, B. The voltage can be set to any desired value by means of a field rheostat. C is the usual platinum gauze cathode about 20 mm. in diameter, which has been accurately weighed. The anode, D, is a platinum wire about 100 mm. long and 1.0 mm. in diameter. It passes through a No. 0 rubber stopper, F, and into the Alundum thimble, I (No. 7338 R.A. 360), which serves as a diaphragm. The stopper is notched, G, to allow gases evolved at the anode to escape. The anolyte, 4% sulfuric acid, is poured into the thimble through the small tube, K. Compressed air to keep the solution stirred enters through tube H.

The electrodes are connected to leads from the motor generator. The beaker containing the solution is placed under the electrodes and 4% sulfuric acid is poured into the Alundum cell through tube K until it begins to overflow through notches G. The overflow is caught in the beaker, which is then raised until the electrodes are immersed in the solution. The block, L, is placed under the beaker. The top of the beaker is covered with split watch glasses, the current turned on, and the voltage adjusted to 2.1 to 2.2. It is important to keep the voltage as close to this value as possible to obtain a bright deposit. Air is bubbled through the solution at a rate which provides the most vigorous stirring without the loss of any electrolyte. The current flowing

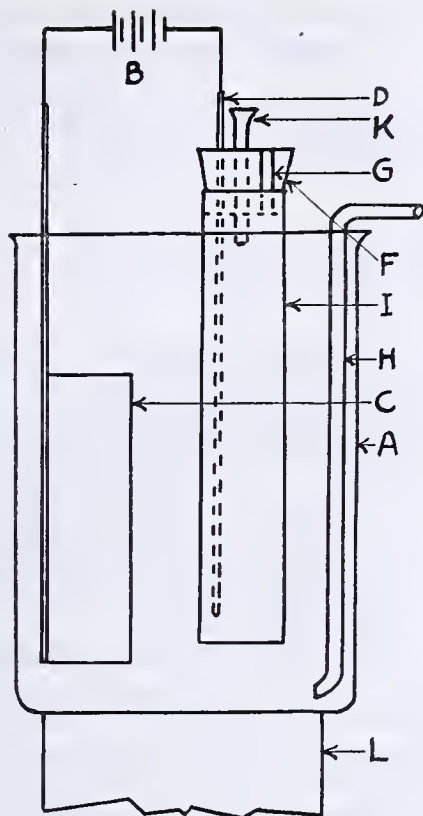


Figure 1

through the circuit is about 0.1 ampere at the beginning and about 0.02 ampere when all the copper is plated out.

The electrolysis is allowed to continue for about 45 minutes before testing the solution to see if all the copper has been deposited. The test used is a variation of the usual sodium diethyldithiocarbamate qualitative test for copper (6). With a glass tube of small bore, a portion of the solution is transferred to a depression on a spot plate. Three drops of concentrated nitric acid are added and the mixture is stirred until the resulting black color disappears. Two drops of concentrated phosphoric acid and then two drops of the sodium diethyldithiocarbamate solution are added. A brown color disappearing almost immediately on stirring to give a colorless spot indicates that all the copper has been plated out. If all the copper has not been plated out, the spot will be yellow in color and will fade after a few minutes. If copper is still present, the electrolysis is continued for another 10 minutes and retested.

When all the copper has been plated out, the watch glasses are removed, the air stirring is cut off, and while the current is still flowing, the block of wood is removed from under the beaker. Then, while the beaker is lowered slowly, the cathode is washed carefully and thoroughly with water from a wash bottle to remove iron salts. The cathode is disconnected, swirled around in a beaker of water for about 10 seconds, dipped in a beaker of methyl alcohol, the excess alcohol is shaken off and the cathode dried in a drying oven maintained at a temperature of 65° C. for 5 to 10 minutes. When the electrode is dry, it is cooled to room temperature and weighed. The weight of the copper deposit times 20 equals the per cent of copper in the sample if a 5.0-gram sample is used.

The procedure for plain cast irons low in copper is the same as for carbon steels. As a rule, more ferric sulfate will be required to oxidize the copper in cast irons than in carbon steels.

On Bureau of Standards samples 115 and 5h, no ferric sulfate was used, since it was necessary to add about 15 drops of nitric acid when the sample was partially dissolved in order to hasten solution and prevent the copper from separating out in large particles. The nitric acid added provided sufficient excess of ferric ion.

#### MODIFICATIONS OF PROCEDURE FOR HIGH-ALLOY STEELS

While the above procedure can be used for the majority of alloy steels, modifications have to be made for some.

With steels containing more than 0.2% molybdenum, a small amount of a substance which is probably a hydrated molybdenum oxide (3) is deposited along with the copper. To get accurate results for such samples, the deposit is stripped with nitric acid and the copper determined colorimetrically as described below. When samples contain about 7% or more of molybdenum, a large quantity of the ferric sulfate solution has to be added before a sufficient excess of ferric ion is obtained. Hence, it is better to add 5 drops of concentrated nitric acid at a time to these samples to oxidize the copper. About 15 to 30 drops are usually required.

High-chromium and 18-8 steels are treated in the same manner as the plain carbon steels, except that when the steels are well decomposed, 5 drops of nitric acid are added to the hot solution and boiling is continued until the sample is dissolved. No ferric sulfate needs to be added to oxidize the copper because the nitric acid used is enough to provide a sufficient excess of ferric ions. The electrolysis is allowed to continue for 45 minutes after a deposit is first noticed. The spot test cannot be used with high-chromium steels, because of the blue color of the solution.

Tungsten steels are run the same as the high-molybdenum steels.

To get accurate copper results for molybdenum, high-chromium, and tungsten steels, the electrode deposit is stripped with nitric acid and the copper determined colorimetrically in the following manner. The deposit is stripped off the electrode in the apparatus shown in Figure 2. A is an open-top, cylindrical separatory funnel, the tube being about 80 mm. long with an inside diameter of 25 mm. B is an ordinary test tube of 16-mm. outside diameter cut down to a height of 120 mm. It is partially filled with water and placed inside the separatory funnel. The electrode, C, is slipped over the test tube, and 1 to 1 nitric acid is poured into the separatory funnel until the deposit is covered by the acid. About 13 cc. of acid are required. The acid is left in contact with the electrode for about one minute, the electrode being moved up and down occasionally to stir the solution. The stopcock is opened and the solution run into a 50-cc. flask. Into the separatory funnel is poured an equivalent volume of 1 to 4 ammonium hydroxide which is run into the same flask after being



in contact with the electrode for a minute. The electrode is washed a second time with dilute ammonium hydroxide. The solution is neutralized with concentrated ammonium hydroxide and 5 cc. are added in excess. It is cooled, diluted to the 50-cc. mark, and shaken well to mix.

A portion of the solution is transferred to the glass cell of a Cenco-Sheard-Sanford Photometer and the intensity of the color developed is measured using a red Corning filter No. 245. The corresponding milligrams of copper present are obtained by referring to a curve constructed by plotting the per cent transmission against the milligrams of copper present in a series of synthetic samples containing known amounts of copper as cupric nitrate (5).

Some typical single results obtained by this method on Bureau of Standards samples are shown in Table I. Duplicate samples check to within 0.01% when copper is below 0.5%.

#### DISCUSSION OF PROCEDURE AND RESULTS

The reaction involved in the oxidation of copper by trivalent iron is



The oxidation is fairly rapid, although not instantaneous. The particles of copper must be in a fine state of subdivision in order to get rapid and complete oxidation. When the steel sample is dissolved as described, this condition is usually automatically met. The only samples encountered in which the copper particles were too large to be oxidized by the regular procedure were standard samples 115 and 5h, both high in copper.

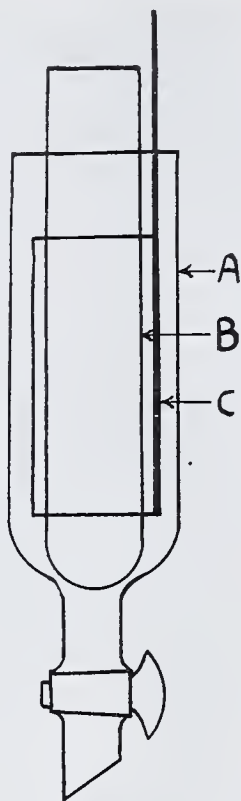


Figure 2

Table II. Effect of Temperature on Copper Deposit

Initial Temp. ° C.	Final Temp. ° C.	Copper Deposited		Color of Deposit
		Electrolytic gravimetric %	Electrolytic colorimetric %	
12	20	0.12	0.12	Bright red
7	18	0.12	0.11	Red
18	23	0.12	0.11	Red
18	22	0.12	0.12	Bright red
34	29	0.13	0.12	Red
34	26	0.13	0.12	Red
78	37	0.14	0.13	Reddish black
74	36	0.14	0.12	Reddish black

Drops of nitric acid can also be used to oxidize the copper. The ferrous ion is oxidized to ferric ion which in turn oxidizes the copper.

It is important to keep the temperature low. When the initial temperature of the solution is high, the deposit will be black in color and the result high. Table II illustrates the effect of temperature on the deposition of copper from a commercial steel containing 0.12% copper.

These results indicate that initial temperatures of 7°, 12°, and 18° C. will give good results. Ice was required to attain temperatures of 7° and 12° C. Since the results obtained at these temperatures were no better than those obtained at 18° C., the solutions were all cooled to 18° C., easily reached by partly immersing the beakers in running tap water about 0.5 hour.

Voltages from 2.0 to 2.6 were studied. All other factors being the same, the copper deposits become darker in color as the voltage is increased and at 2.6 volts are black. However, the accuracy of the method is not appreciably altered. The voltage employed does not change to any appreciable extent the time required for electrolysis. With voltages 2.0 and below, the copper does not plate out or does so very slowly. Hence, the importance of maintaining a voltage of 2.1 to 2.2 is obvious.

About 40 to 45 minutes are required to plate the copper from an ordinary carbon steel whose copper content is 0.3% or less. When the copper content is higher than 0.3%, a correspondingly longer time is required. Ordinarily, the deposition will be noticeable in from 1 to 5 minutes, but if too large an excess of ferric ion is present at the start, it will take longer.

For plain carbon steels and cast irons, the deposit will usually be a darkish red color. Some deposits will be bright red in color while others almost black. However, regardless of color the results will be satisfactory. Copper deposits from alloy cast irons and molybdenum steels will usually be a dull black. Those from samples containing much tungsten or chromium will be black with a bluish tinge.

The principal sources of error in this method are incomplete oxidation of the copper by the ferric sulfate treatment, diffusion of the copper ions into the anolyte, absorption of copper ions by the Alundum cell, and copper remaining in the catholyte. These sources of error were investigated and in only a few instances was as much as 0.1 mg. of copper found in any one source.

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Table I. Determination of Copper in Iron and Steel

Bureau of Standards Sample No.	Copper, Standard Value, %	Copper, Electro-lytic-Gravimetric, %	Copper, Electro-lytic-Colorimetric, % <sup>a</sup>	Type of Steel
13c	0.165	0.16	0.16	Carbon steel
14c	0.025	0.03	0.03	Carbon steel
19c	0.161	0.17	0.16	Carbon steel
20d	0.165	0.16	0.16	Carbon steel
35a	0.267	0.26	0.26	Carbon steel
65b	0.20	0.20	0.20	Carbon steel
129	0.166	0.17	0.17	Carbon steel
16c	0.060	0.06	0.06	Carbon steel
74	0.029	0.04	0.04	Cast iron
107	0.074	0.08	0.07	Cast iron, Ni = 0.807, Cr = 0.455
107	0.074	0.09	0.08	Cast iron, Ni = 0.807, Cr = 0.455
115 <sup>b</sup>	6.44	6.40	6.45	Cast iron, Ni = 15.89, Cr = 2.17
115 <sup>b</sup>	6.44	6.45	6.45	Cast iron, Ni = 15.89, Cr = 2.17
115 <sup>b</sup>	6.44	6.60	6.45	Cast iron, Ni = 15.89, Cr = 2.17
115h	1.46	1.41	...	Cast iron
100	0.124	0.13	0.13	Mn = 1.38
33b	0.114	0.12	0.11	Ni = 3.48
73	0.033	0.04	0.04	Cr = 13.93
32b	0.117	0.11	0.11	Cr = 0.638, Ni = 1.21
30c	0.099	0.11	0.10	Cr = 0.997, V = 0.235
72b	0.100	0.11	0.11 <sup>c</sup>	Cr = 0.46, Mo = 0.224
36	0.110	0.16	0.11 <sup>d</sup>	Cr = 2.32, Mo = 1.01
111	0.122	0.13	0.13	Ni = 1.75, Mo = 0.215
106	0.142	0.15	0.15	Cr = 1.29, Mo = 0.164, Al = 1.06
101A	0.051	0.06	0.05	Cr = 18.33, Ni = 8.99
132	0.15	0.28	0.15	Mo = 7.1, W = 6.3
50a	0.074	0.12	0.07	Cr = 4.09, V = 1.64
				Cr = 3.52, V = 0.97, W = 18.25

<sup>a</sup> Values obtained by stripping electrodes of copper shown in adjacent column and determining copper as described in procedure.

<sup>b</sup> 0.5-gram sample used.

<sup>c</sup> On replating, 0.10% copper was obtained.

<sup>d</sup> On replating, 0.11% copper was obtained.



# Determination of Vitamin A and Carotenoids in Butterfat

## Comparison of Direct Spectrophotometry with Filter Photometry and Use of the Antimony Trichloride Reaction

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THE data reported herein were obtained during a comparative study of methods for the determination of vitamin A and carotenoids in butter by the Technical Committee on Vitamin A Researches in cooperation with the National Cooperative Project on the vitamin A Potency of Market Butters.

This committee consisted of:

L. A. Maynard, U. S. Nutrition Laboratory, Ithaca, N. Y., chairman

C. J. Koehn, Alabama Polytechnic Institute, Auburn, Ala., referee

C. A. Cary, U. S. Department of Agriculture, Bureau of Dairy Industry, Beltsville, Md.

L. S. Palmer, Minnesota Agricultural Experiment Station, St. Paul, Minn.

W. H. Peterson, Wisconsin Agricultural Experiment Station, Madison, Wis.

H. R. Guilbert, California Agricultural Experiment Station, Davis, Calif.

I. L. Hathaway, University of Nebraska, Dairy Department, Lincoln, Neb.

F. P. Zscheile, Purdue University Agricultural Experiment Station, Lafayette, Ind.

This committee was established by a committee of the state experiment stations, which cooperated with C. H. Bailey as chairman. The general problem was suggested by the Committee on Food and Nutrition of the National Research Council.

Six representative samples of butterfat from sweet cream were prepared by the referee and sent to the various collaborators, each of whom analyzed them by a method previously agreed upon and reported results independently to the referee. In this laboratory, the authors have investigated the possible use of direct spectrophotometry for this determination because excellent equipment is available and such a method offers prospects of greater brevity, owing to fewer laboratory manipulations.

This paper compares the results of the direct spectroscopic method and those obtained by colorimetry. The analytical results on the butterfat samples are taken from the report of the referee (7).

### EXPERIMENTAL

The following directions for preparation of the extract are essentially those given in reports of the Technical Committee on Vitamin A Researches (6, 7).

Weigh a 10-gram sample of filtered butterfat (liquid or solid) or of crude butter (solid) into a 300-ml. saponification flask. Add a mixture of 5 ml. of saturated aqueous potassium hydroxide and 20 ml. of aldehyde-free methanol. Connect a reflux condenser with a ground-glass joint to the flask and boil the mixture on a hot plate for 10 minutes. Dilute immediately with 40 ml. of distilled water and cool to room temperature under the tap.

Transfer to a separatory funnel, using 50 ml. of additional water to rinse the flask. Add 100 ml. of peroxide-free diethyl ether to extract the nonsaponifiable fraction. The ether should give a negative Jorissen test (2). After separation of the two phases, extract the aqueous layer three more times, each time with a 50-ml. portion of ether. Combine the ether extracts and wash with distilled water until free of alkali (4 to 6 times). Reduce the volume of ether to about 80 ml. on a steam bath, recovering the ether with a condenser. Dry over anhydrous sodium sulfate and make to volume of 100 ml. Place in refrigerator if spectroscopic observations cannot be made at once.

Make spectroscopic observations the same day if possible; this is especially desirable for the ultraviolet readings. Use wave lengths 3240 and 4370 Å. for vitamin A and total carotenoids,

respectively. Be certain to employ for the solvent ( $I_0$ ) cell ether that came from the same bottle as that used for the preparation of the extract. This is important to avoid errors due to absorption in the ultraviolet by solvent impurities. Methanol of a good grade must be used and an occasional blank determination is advisable to avoid spurious results due to impurities from the methanol.

Table I. Summary of Observations on Butterfats by Direct Spectrophotometry

Determined 2-26-42 to 3-19-42 Churning date	Sample No.	$E_{1\text{ cm.}}^{1\%} \times 1000$			
		3260 Å.	4370 Å.	4525 Å.	4675 Å.
10-18-39	1	15.8	20.8	24.5	20.7
1-6-41	2	11.0	8.60	9.84	8.50
7-11-41	3	16.8	26.0	30.0	25.7
10-17-41	4	11.7	17.5	21.0	17.8
11-21-41	5	10.5	13.8	16.9	14.0
12-31-41	6	8.71	7.35	8.85	7.38
Redetermined (6-10-42)		3240 Å.			
	1	15.2	20.9	24.4	20.8
	2	10.8	8.61	9.80	8.45
	3	16.4	24.7	28.6	24.6
	4	11.7	17.7	20.9	17.6
	5	10.8	13.8	16.3	13.9
	6	7.86	7.30	8.71	7.27

The spectroscopic methods and instrument were those employed earlier in a study of the effect of solvent on the spectrum of vitamin A (13). However, a sample of crystalline vitamin A alcohol with a still higher absorption value has since been made available through the courtesy of J. G. Baxter of Distillation Products, Inc. The same preparation for which an extinction coefficient of 1780 at the absorption maximum of a solution in ethanol was reported (3) had a corresponding  $E_{1\text{ cm.}}^{1\%}$  value (13) of 1825 at 3240 Å. in diethyl ether solution. This value was used as the standard for calculation of the vitamin A content of butterfat. The absorption curve for this preparation was parallel to the curve for sample B-210 (13) over the region of maximum absorption, but the slope between 3400 and 3800 Å. was steeper than that given for the ether or ethanol solutions of sample B-210.

The procedure for the antimony trichloride method is almost identical with the above up to the preparation of the ether extract. It continues (6, 7) with a division of the sample into two parts for determination of carotenes and vitamin A separately.

1. CAROTENE DETERMINATION. Carefully evaporate the ether solution to dryness, dissolve the residue in Skellysolve B, extract with aqueous methanol or diacetone alcohol, dry with sodium sulfate, and measure the transmission in the 440  $m\mu$  region with a photoelectric filter photometer.

2. VITAMIN A DETERMINATION. Evaporate the other aliquot of the ether extract to dryness, dissolve the residue in chloroform, develop the color by treatment with the antimony trichloride reagent added under carefully controlled conditions, and determine the transmission in the region of 620  $m\mu$  with the same photoelectric filter photometer. This transmission reading must be corrected for the reaction of carotene with the reagent.

This method is very similar to that described in detail by Koehn and Sherman (8).

Table I presents the more pertinent numerical data obtained on the simple ether extracts by direct spectrophotometry. Determinations at 3240 and 3260 Å. are only slightly different but



measurement at 3240 Å. is preferred because the absorption maximum of pure vitamin A was found at this wave length.

The spectra of beta-carotene and of neo-beta-carotene, prepared by adsorption on alumina (4) were determined from 3200 to 5100 Å. in diethyl ether solutions (14). [This neo-beta-carotene probably consists largely of the isomer designated by Polgár and Zechmeister (11) as neo-beta-carotene B.] Significant quantitative differences in both wave length and intensity of absorption were found between these absorption curves and those presented for the same pigments in hexane solution (4). The curves for the two pigments in ether solution are coincident at 4370 Å. (14); hence this wave length is best chosen for analysis of total carotenoids. The  $E_{1\text{cm}}^{1\%}$  value at 4370 Å. is 2040.

A preliminary analysis indicated an appreciable content of carotenols (ca. 20%) in these butters (7). (The use of this term has been discussed previously, 12.) Further study led to the final recommendation of the committee (6) that for carotene determination the residue from evaporation of the ether extract be dissolved in Skellysolve B and extracted with either 92% aqueous methanol or 94% aqueous diacetone alcohol to remove the carotenol fraction. The use of diacetone alcohol was recommended for butters produced from cows fed acid or molasses silage, in which cases considerably more pigment than otherwise is produced that is not beta-carotene. These pigments are removed with the carotenols more effectively by diacetone alcohol than by methanol (5).

In this laboratory a comparative study was made between the use of methanol and the use of diacetone alcohol on these six butterfats. The ether extract was evaporated almost to dryness on the steam bath and the residue was dissolved in 100 ml. of hexane and divided into two aliquots of 50 ml. each. Each aliquot was extracted four times with 25-ml. portions of hypophasic solvent to remove the carotenols. The hexane solution was then analyzed spectrophotometrically at wave lengths 4360, 4780, and 4850 Å. for beta-carotene and neo-beta-carotene (4). The results at 4360 Å. indicated that the diacetone alcohol procedure caused the removal of 6 to 9% more carotenoid in the carotenol fraction than did the use of methanol. The carotenol content by the methanol method varied from 15 to 28% of the total carotenoids. The carotenol contents are much higher than any discussed recently by Morton (9).

Characteristic curves (log log  $I_0/I$  vs. wave length) of the carotene fraction in hexane agreed much better with those of assumed beta-carotene-neo-beta-carotene mixtures than did those of the total carotenoids in ether solution. Agreement was not complete, however; this is reflected in the lack of agreement (in most samples) between analytical results for neo-beta-carotene at the two wave lengths 4780 and 4850 Å. (differences of 0.6 to 7.0%). Values ranged from 70 to 86% neo-beta-carotene. The content of this isomer may well have been increased by the hot saponification procedure and other isomers may have been formed (11). When the hexane solution of the carotene fraction was chromatographed on alumina and developed with 20% ether-80% hexane, a zone was found which contained a pigment with a spectrum resembling that of neo-beta-carotene but with increased absorption in the region below 4300 Å.

## COMPARISON OF CAROTENOID CONTENTS

Table II presents the analyses for total carotenoids (7) as determined on the same butterfats at seven laboratories, with the respective deviations from the mean for each sample.

It is evident that from any practical point of view the mean absorption deviations of all collaborators were comparatively small. From the total of 42 determinations, only 5 deviated from their corresponding means by more than 7%, the maximum deviation being 13.3%. The deviations from certain laboratories are all or nearly all either positive or negative. This may be due to the use of carotene standards of slightly different degrees of purity, since each laboratory provided its own standard. In general, agreement among the various laboratories is very satisfactory when the difference in standards is considered. The results from this laboratory have both the smallest mean absolute deviation and the smallest maximum deviation, each approximately one third the magnitude of the over-all mean absolute deviations.

## COMPARISON OF VITAMIN A CONTENTS

Table III presents the vitamin A contents of the same butterfat samples as reported by four collaborators (7), all of whom employed crystalline vitamin A alcohol as reference standards and reported in terms of weight units. These results are in much better agreement among themselves than those from four collaborators who used U.S.P. reference cod liver oil as a standard (5) and reported results in International Units of Vitamin A. These latter results will not be considered here.

Since the results from this laboratory were the only ones obtained by direct spectrophotometry, they are compared with the means of the results obtained at the other three laboratories. Corresponding deviations are given. The uncorrected results from Purdue were calculated from the total absorption observed at 3260 Å. ( $E_{1\text{cm}}^{1\%}$  for vitamin A in ether = 1825) on the ether extract. Because the characteristic curve of the extract did not agree well with that of pure vitamin A alcohol, a correction is necessary for absorption due to carotenoids (14), and other contaminants of unknown nature. The ratios of the uncorrected values to the corresponding means from the other three laboratories were fairly constant ( $\pm 4\%$ ). As a mean value, a correction factor of 0.78 ( $= \frac{1}{1.28}$ ) was then applied to the uncorrected values (Table III) for miscellaneous absorption of substances other than vitamin A.

Among the 18 results from the collaborators using the colorimetric method, deviations from the mean are 8.15% or less, with

Table II. Contents of Total Carotenoids and Deviations from Mean

Total Carotenoids, Micrograms per Gram of Butterfat								
Sample No.	Purdue <sup>a</sup>	Minnesota	Wisconsin	California	U.S.D.A. <sup>a</sup>	Ithaca	Alabama	Mean
1	10.2	10.50	9.95	10.65	9.74	10.3	10.46	10.25
2	4.21	4.14	4.02	4.30	3.75	4.2	4.34	4.13
3	12.7	12.96	12.15	12.81	11.68	12.2	12.67	12.45
4	8.56	8.92	8.45	7.37	8.50	8.7	9.00	8.50
5	6.77	7.22	6.65	6.26	7.00	6.4	7.00	6.76
6	3.60	3.65	3.66	3.55	3.33	3.6	4.05	3.64
Deviations from Mean								
	%	%	%	%	%	%	%	Mean Absolute Deviation
1	-0.49	+2.44	-2.93	+3.90	-4.97	+0.49	+2.05	2.47
2	+1.93	+0.24	-2.67	+4.12	-9.20	+1.70	+5.09	3.56
3	+2.00	+4.09	-2.41	+2.89	-6.18	-2.01	+1.77	3.05
4	+0.70	+4.94	-0.59	-13.3	0.00	+2.36	+5.89	3.97
5	+0.15	+6.80	-1.63	-7.40	+3.55	-5.33	+3.55	4.06
6	-1.10	+0.27	+0.55	-2.48	-8.52	-1.10	+11.3	3.62
Mean absolute deviation	1.06	3.13	1.80	5.68	5.40	2.16	4.95	3.46
Maximum deviation	+2.00	+6.80	-2.93	-13.3	-9.20	-5.33	+11.3	7.25
<sup>a</sup> Determinations with photoelectric spectrophotometer. All others with photoelectric filter photometer								
Values from U.S.D.A. revised (by private communication) from those reported earlier (7), following discovery of systematic error in earlier results.								

<sup>a</sup> Determinations with photoelectric spectrophotometer. All others with photoelectric filter photometer. Values from U.S.D.A. revised (by private communication) from those reported earlier (7), following discovery of systematic error in earlier results.



Table III. Vitamin A Contents by Colorimetric Method and Deviations from Mean  
(Comparison with direct spectrophotometry)

Sample No.	Vitamin A, Micrograms per Gram of Butterfat				Purdue <sup>a</sup>		Ratio (Uncorrected to Average)
	Minnesota	Wisconsin	Alabama	Mean	Uncorrected	Corrected	
1	6.23	6.81	6.56	6.53	8.65	6.75	1.33
2	4.54	4.92	5.03	4.83	6.03	4.70	1.25
3	6.78	6.94	7.61	7.11	9.21	7.18	1.29
4	4.85	4.88	5.00	4.92	6.42	5.01	1.30
5	4.56	4.63	4.77	4.66	5.76	4.50	1.24
6	3.38	3.70	3.94	3.68	4.77	3.72	1.29
						Mean	1.28
	Deviations from Mean			Mean Absolute Deviation			
	%	%	%				
1	-4.60	+4.29	+0.46	3.12	....	+3.37	....
2	-6.00	+1.86	+4.14	4.00	....	-2.77	....
3	-4.64	-2.39	+7.02	4.68	....	+0.98	....
4	-1.42	-0.81	+1.62	1.28	....	+1.83	....
5	-2.15	-0.64	+2.36	1.72	....	-3.43	....
6	-8.15	+0.54	+7.06	5.25	....	+1.08	....
Mean absolute deviation	4.49	1.76	3.77	3.34	....	2.24	....
Maximum deviation	-8.15	+4.29	+7.06	5.25	....	3.43	....

<sup>a</sup> Determinations based on direct photoelectric spectrophotometry at 3260 Å. with standard  $E_{1\text{ cm.}}^{1\%}$  value of 1825. All others based on colorimetric reading with photoelectric filter photometer. Crystalline vitamin A alcohol used as standard in all cases.

an over-all mean absolute deviation of 3.34%. The corrected results from direct spectrophotometry have mean absolute and maximum deviations two thirds as great as the over-all averages. Unsuccessful attempts were made to improve the agreement by correction for the amounts of carotenoids present. Probably no better agreement could be expected, nor would it be of significant practical value in butter studies.

INFLUENCE OF AZO DYES

Since methods agreed upon by the Technical Subcommittee on Vitamin A Researches (6) were to be applied to a survey of commercial butters, it was necessary to investigate the possible effects of artificial coloring materials upon the analytical results. The three substances most commonly employed for this purpose are annatto and the azo dyes (certified as F. D. and C Yellow Nos. 3 and 4.

Annatto is easily eliminated from the original ether extract, since it is contained in the aqueous methanol phase (10). The two closely related azo dyes are not so removed but do not interfere with the colorimetric method involving the antimony trichloride reaction (6). With the method of direct spectrophotometry, the situation is very different (10).

These azo dyes have almost identical absorption spectra, with two broad maxima in the region measured (3100 to 9400 Å.). These occur at 3450 and 4450 Å. with a minimum at 3800 Å. Absorption is not reduced to 10% of its maximum value until 5200 Å. toward the red. On the specific basis, these curves are considerably lower than those of either vitamin A or beta-carotene. On the molecular basis, the ratio of the maximum absorption coefficients of beta-carotene to those of the dyes is approximately 9 to 1.

The absorption curves of the azo dyes differ sufficiently in shape from those of beta-carotene and of total carotenoids from many butters to permit analysis for the dye content by application of simultaneous equations to the absorption data, if a standard curve could be used to represent the carotenoid fraction. This technique is, however, of very limited application, because the characteristic curves of the carotenoids of butters from silage-fed cows cannot be interpreted from spectroscopic data available at the present time and absorption properties may change during storage of butters (14).

The azo dyes are quantitatively removed in the carotenol fraction by the extraction procedure with aqueous methanol or

diacetone alcohol. The carotenes may be accurately estimated in the resulting hexane solution, but part or all of the vitamin A is extracted by the hypophase. Therefore, an attempt was made to determine the dyes independently and to estimate the vitamin A concentration after application of corrections for absorption by the dyes to the total absorption in the original ether solution at 3240 Å. This attempt was based on recovery of the dyes from the carotenol fraction. Efforts to separate the dyes from the carotenols by solvent extraction were unsuccessful. Application of the extraction procedure with sulfuric acid, as outlined by the Association of Official Agricultural Chemists (1) proved fruitless, since it was not possible

to recover the dyes quantitatively from the acid solution because of decomposition of the dye. Variation of the strength of acid used and substitution of hydrochloric acid led to no better results.

Since the presence of these azo dyes does not interfere seriously, the antimony trichloride reaction (6) is the preferred physico-chemical method available for butters containing such dyes.

ACKNOWLEDGMENT

The writers wish to thank the technical subcommittee for making available the data of its reports (6, 7). They are indebted to Referee C. J. Koehn for certain suggestions and for a supply of the artificial coloring materials studied.

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# Quantitative Determination of Nicotine and Nornicotine in Mixtures

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A method for the determination of nicotine and nornicotine in mixtures is based on the differences in the colors produced by these substances when reacted with cyanogen bromide. Typical results of application of this procedure to tobacco analysis are given.

IN RECENT years, Markwood (11) has called attention to the fact that certain strains of *Nicotiana tabacum* may contain relatively large amounts of nornicotine. Although such "high-nornicotine" strains probably constitute only a small minority of those generally used by man, it seems safe to assume that virtually all tobacco contains some nornicotine.

The generally used silicotungstic acid method for the determination of nicotine (1) fails to distinguish between nicotine and nornicotine, and an incomplete and variable portion (ca. 25 to 40%) of the nornicotine present in tobacco will appear in the nicotine analysis as apparent nicotine. Since the toxic and other pharmacologic properties of nornicotine differ considerably from those of nicotine, and since its per cent transfer from tobacco to smoke differs markedly from that of nicotine, this situation contributes to the difficulty of evaluating the potential effects of tobacco on the smoker. Accordingly, methods for the routine determination of nornicotine as well as more accurate methods for the determination of nicotine are desirable.

Within recent months, Markwood (10) and Bowen and Barthel (4) have proposed methods for the chemical separation and determination of nicotine and nornicotine which eliminate the possibility of including nornicotine in the nicotine analysis as apparent nicotine. Both methods divide nicotine and its related steam-volatile alkaloids into two groups, the division being based on whether or not the nitrogen of the group substituted in the pyridine ring will react with nitrous acid to form a nitroso compound. Since nicotine belongs to one group and nornicotine to the other, this effectively segregates the two for analytical purposes. While the specificity of this procedure does not go beyond this, since nicotine and nornicotine are seemingly the two chief alkaloids of tobacco, it represents a distinct advance in tobacco analysis.

During investigations on the fate of nicotine (7) and nornicotine (8) in the animal organism, the authors noted that when cyanogen bromide was added to dilute solutions of nicotine a pale yellowish green color developed, and that when cyanogen bromide was added to solutions of nornicotine, under certain conditions, a red color developed. The cyanogen bromide reaction with nicotine was utilized by Barta and Marschek (2) and Markwood (9) for the determination of nicotine, but they employed in addition an alcoholic solution of  $\beta$ -naphthylamine which converts the yellowish green of the nicotine-cyanogen bromide reaction to a pink or red color. This destroys the usefulness of the reaction for distinguishing between nicotine and nornicotine. In the present study, the authors have examined the conditions involved in the formation of color by nicotine and nornicotine when reacted with cyanogen bromide, and have formulated a method for quantitatively determining these substances in mixtures without preliminary separation.

## METHOD

**REAGENTS.** Sodium chloride, 10 *M* sodium hydroxide, 0.2 *N* sulfuric acid, 0.2 *N* potassium hydroxide, and 1.67 *M* potassium dihydrogen phosphate, prepared fresh for each day's analyses.

Sodium cyanide, 2 *M*, 0.2 *M*, and 0.02 *M* prepared fresh for each day's analyses.

Cyanogen bromide reagent, prepared for each day's analyses by titrating 25 ml. of cold saturated bromine with 2 *M* sodium cyanide to almost disappearance of color and then titrating to complete disappearance of color with 0.2 *M* sodium cyanide, avoiding an excess. The solution is brought to room temperature and, with the aid of a glass titration electrode, adjusted to pH 7.0 with 0.02 *M* sodium cyanide, and then to pH 4.1 to 4.2 with 0.2 *N* sulfuric acid.

Nicotine standard containing about 20 micrograms per ml., adjusted to pH 4.1 to 4.2 with 0.2 *N* sulfuric acid.

Nornicotine standard containing about 60 micrograms per ml., adjusted to pH 4.1 to 4.2 with 0.2 *N* sulfuric acid.

**PREPARATION OF TOBACCO DISTILLATE.** A 0.5- to 2.0-gram sample of finely ground tobacco is placed in a 300-ml. Kjeldahl flask, 10 grams of sodium chloride (4), 10 ml. of 10 *M* sodium hydroxide, and a little paraffin are added, and the mixture is steam-distilled to a distillate volume of about 800 ml. in 60 minutes. (Three milliliters of 0.2 *N* sulfuric acid plus enough distilled water to cover the condenser opening are added to the receiving flask prior to distillation and the fluid volume in the Kjeldahl during distillation is maintained at 50 to 75 ml.) The distillate is then adjusted to pH 4.1 to 4.2 with 0.2 *N* sulfuric acid or potassium hydroxide and the volume made up to 1 liter.

Since completion of the data presented here the authors have adopted the distillation technique described by Bowen and Barthel (3). In addition to saving time, since the method described below is capable of measuring very small quantities of nicotine and nornicotine, this distillation is preferable when little material is available.

**COLOR DEVELOPMENT.** Two aliquots of 6 ml. or less of the tobacco distillate (the volume used should by prediction contain not more than 80 micrograms of nicotine and 150 micrograms of nornicotine) are pipetted into 20  $\times$  150 mm. test tubes and the total volume in each tube is brought to 6 ml. with distilled water. To each tube are added 2 ml. of 1.67 *M* potassium dihydrogen phosphate.

The tubes are then treated individually as follows: A tube is placed in an 80° C. water bath for exactly 5 minutes. It is then removed, 2 ml. of cyanogen bromide reagent are added, mixed by agitation, and the tube is returned to the water bath (this step is completed in exactly 15 seconds). The tube is allowed to remain in the water bath for 2 minutes and 45 seconds and is then removed and quickly placed in an ice-water bath for

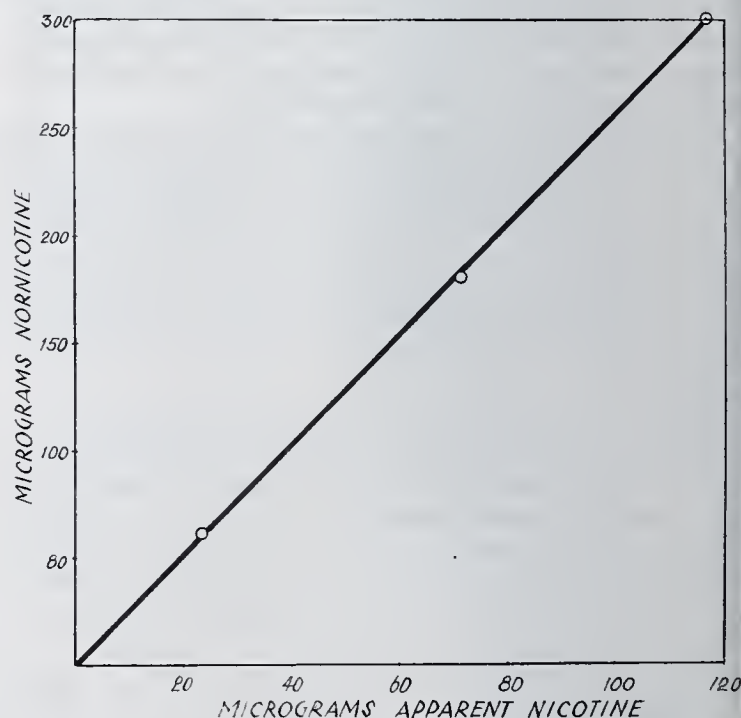


Figure 1. Interference of Nornicotine in Determination of Nicotine by Cyanogen Bromide Method



1 minute. The contents are transferred to a spectrophotometer (the authors have used the Coleman 10S model with a 30-millimicron slit) cuvette and the intensity of the color developed by nicotine is read at 540 millimicrons and that developed by nornicotine at 375 millimicrons. This operation should be completed within 4 minutes following removal of the tube from the ice-water bath, during which time the next tube can be completing the 5-minute preheating period.

Since tobacco distillates are often slightly colored, it is well prior to the color determination to balance the spectrophotometer to 100% transmission against a solution containing a volume of distillate equal to that used for color development diluted to 10 ml. with distilled water.

The nicotine and nornicotine values corresponding to the color intensities determined are found by consulting calibration curves for nicotine and nornicotine prepared from standard solutions as described below, and the remaining calculations completed.

**PREPARATION OF CALIBRATION CURVES.** Aliquots of the nicotine standard (1-, 3-, and 5-ml.) are treated as above for color development, intensities being read at 375 millimicrons. One tube containing 6 ml. of distilled water plus the phosphate solution is similarly treated to provide a blank on the cyanogen bromide reagent. The nicotine calibration curve is then prepared by plotting the data so obtained on semilog paper, per cent transmittance being plotted on the logarithmic axis. A straight-line curve will result.

Color is similarly developed for 1-, 3-, and 5-ml. aliquots of the nornicotine standard and intensities are read at 540 and 375 millimicrons. The cyanogen bromide reagent gives no blank at this wave length. The calibration curve for nornicotine is then prepared in the manner described above for nicotine, using the data obtained at 540 millimicrons. Again a straight-line curve will result.

The color developed by nornicotine in this reaction follows Beer's law in the presence of the nicotine color. Accordingly, the values obtained from the nornicotine calibration curve for the nornicotine contents of tobacco are true. However, the color developed by nornicotine has an additive effect on the intensity of the nicotine color (Figure 2, D). To determine the degree, the spectrophotometer readings obtained with the nornicotine standard at 375 millimicrons are converted to micrograms of apparent nicotine by use of the nicotine calibration curve. By plotting graphically the nornicotine values versus their apparent nicotine equivalent a straight-line relationship should be obtained (see Figure 1). Since the true amount of nornicotine in mixtures of nornicotine and nicotine can be obtained directly by consulting the nornicotine calibration curve, it becomes possible to evaluate the true nicotine content of the mixture by subtracting from the apparent nicotine content the amount due to nornicotine.

These calibration curves need not be redetermined for each day's analyses; however, it is advisable to check them with standard solutions of nicotine and nornicotine whenever a new source of bromine water, sodium cyanide, or potassium dihydrogen phosphate is used.

#### FACTORS INFLUENCING COLORS DEVELOPED

**INORGANIC SALTS.** Figure 2, A, shows the transmittance curve of the color produced by reacting nicotine, in water solution brought to pH 4.1 with sulfuric acid, with cyanogen bromide. Minimum transmittance occurs at 385 millimicrons. Inorganic salts markedly influence the rate of development, intensity, and stability of the color developed in this reaction (Figure 3). Thus sodium bicarbonate accelerates the rate of development of the color and increases its maximum intensity. All the other salts studied tended to slow the rate of development of color, to increase its stability, and, depending on the individual salt and the amount used, to increase or decrease the maximum intensity of the color developed. When 0.40 gram of potassium dihydrogen phosphate is added to the nicotine-cyanogen bromide reaction mixture the minimum for the color developed is shifted to 375 millimicrons (Figure 2, B).

Figure 2, C, shows the transmittance curve of the color produced by reacting nornicotine, in water solution brought to pH 4.1 with sulfuric acid, with cyanogen bromide. Minimum

transmittance occurs at 390 millimicrons. Presence of inorganic salts can profoundly affect the color developed in this reaction. Phosphate ion, ammonium ion, or molybdate ion, for example, completely alters the transmittance curve of the color produced, creating a minimum at 540 millimicrons, while retaining a component in the deep blue (Figure 2, D). Sodium chloride, potassium chloride, or sodium sulfate in amounts sufficient to saturate the reaction mixture also tend to produce the same change but are much less potent and fail to stabilize the reaction, the red color fading rapidly.

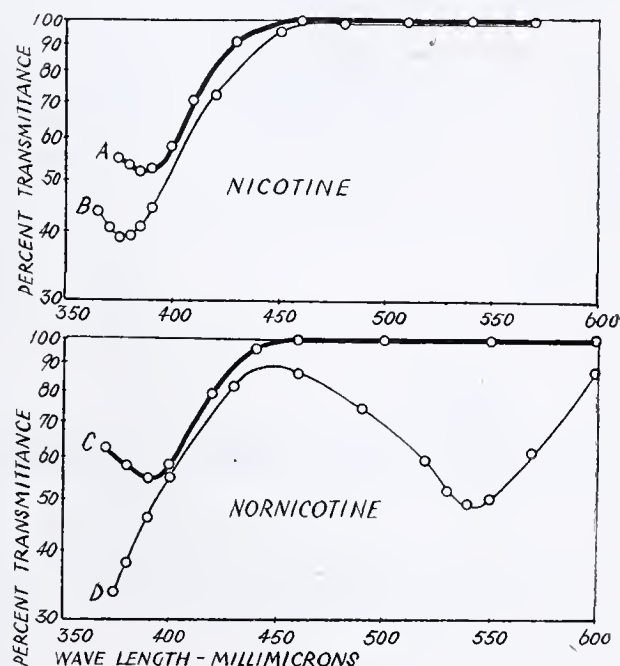


Figure 2. Development of Color

A and C, absorption curves of colors produced by reacting nicotine and nornicotine in water solution brought to pH 4.1 with sulfuric acid, with cyanogen bromide. B and D, effect on absorption curves of adding 0.4 gram of potassium dihydrogen phosphate to reaction mixture.

The potassium dihydrogen phosphate is added to the reaction mixture in the method described to separate widely the minima of the colors developed by nicotine and nornicotine and to increase their stability.

The necessity for excluding extraneous salts from the reaction mixtures is evident. The small amount of sulfuric acid used in adjusting pH does not appear materially to affect the intensity of the colors developed. Unfortunately, the amount of cyanide used, over and above that needed to decolorize the bromine, in preparation of the cyanogen bromide reagent, is considerably more critical. The procedure adopted for the preparation of the cyanogen bromide reagent takes advantage of the fact that the pH change of the reagent during titration with sodium cyanide is markedly accelerated when an excess of sodium cyanide has been reached. By bringing the reagent to a definite pH in this titration a fairly constant excess of cyanide is obtained permitting reproducible development of color.

**TEMPERATURE.** When the nicotine and nornicotine reactions with cyanogen bromide are carried out in the presence of phosphate at room temperature they proceed at unequal rates, the nornicotine color having reached the maximum intensity and begun to fade when the nicotine color is just approaching its maximum intensity. While this is unimportant when determining nicotine or nornicotine individually in pure solutions, it becomes a complicating factor when mixtures are used. To circumvent this, the authors have made use of the color-stabilizing action of phosphate.

When the colors are developed at room temperature, the optimum amount of potassium dihydrogen phosphate to permit moderate stabilization of color while retaining near optimum intensity of color development is about 0.10 gram (for nicotine see



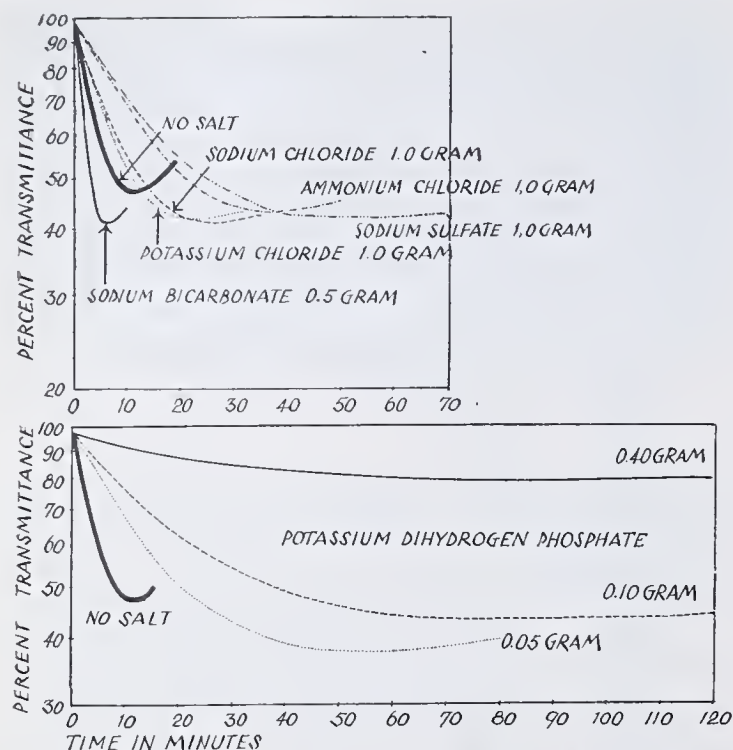


Figure 3. Effect of Salts on Color Produced by Reacting Constant Amounts of Nicotine with Cyanogen Bromide at Room Temperature

Figure 3). Larger amounts, achieving greater stability, can be employed without serious decrease in the intensity of color developed, provided the reactions are carried out at elevated temperature. For this purpose 80° C. was arbitrarily chosen.

The optimum time for development of color at 80° C. for nornicotine is shown in Figure 4, A, and for nicotine in Figure 4, C. For both substances, maximum color is developed in about 3 minutes; if the reaction mixture is held longer at 80° C. the colors rapidly fade. Rate of fading is greatly reduced by removing the tube containing the reaction mixture from the 80° C. water bath at exactly 3 minutes and immediately placing it in an ice-water bath for 1 minute prior to determining the intensity of the color developed. Figure 4, B and D, shows the rate of fading of these colors when chilled to room temperature at the end of their 3-minute period of development.

Table I. Nicotine and Nornicotine Content of Composite Samples of Cigaret Tobacco

Type of Tobacco	A.O.A.C. Distillation				Distillation from 10 Grams of Sodium Chloride Plus 10 Ml. of 10 M Sodium Hydroxide			
	Cyanogen Bromide Method	Nornicotine, %	Nicotine, %	Difference, %	Cyanogen Bromide Method	Nornicotine, %	Nicotine, %	Difference, %
Turkish	0.045	0.74	0.81	0.02	0.11	0.71	0.87	0.04
Maryland	0.11	1.32	1.52	0.08	0.29	1.34	1.68	0.07
Bright	0.13	1.73	2.01	0.14	0.27	1.72	2.18	0.17
Burley	0.23	2.45	2.81	0.11	0.48	2.47	3.09	0.10

\* Nicotine by silicotungstic acid precipitation minus sum of nicotine plus nornicotine by cyanogen bromide method corrected for difference in molecular weight of nicotine and nornicotine.

The optimum amounts of potassium dihydrogen phosphate to be used when the reactions are carried out at 80° C. are shown in Figure 5. Maximum color from the nornicotine-cyanogen bromide reaction develops in the presence of about 0.4 gram and from the nicotine-cyanogen bromide reaction in the presence of 0.075 gram. Since for equal amounts of nicotine and nornicotine, the intensity of color developed by the nicotine-cyanogen bromide reaction is considerably greater than that developed by the nornicotine-cyanogen bromide reaction, values for mixed solutions have always been determined at the potassium dihydrogen phosphate optimum for nornicotine.

pH. The effect of pH on the intensity of the colors developed is shown in Figure 6. Under the conditions of temperature and

phosphate content adopted, a sharp maximum develops for both nicotine and nornicotine at about pH 4.1 to 4.2. In preparing the solutions used in this experiment the phosphate was added prior to adjustment of pH. In practice, when the phosphate is added to unbuffered solutions of nicotine and nornicotine, preliminary adjustment of pH is not so critical as Figure 6 would indicate, since the potassium dihydrogen phosphate tends to produce a constant pH which is near optimum.

Table II. Nicotine and Nornicotine Content of Maryland High-Nornicotine Tobacco

Type of Distillation	Type of Analysis	A	Specimen B	C	D
A.O.A.C.	Nicotine, %, by A.O.A.C. silicotungstic acid method	0.76	1.77	2.14	0.27
Distillation from 10 grams of sodium chloride plus 10 ml. of 10 M sodium hydroxide	Nicotine, %, by cyanogen bromide method	0.37	1.00	1.63	0.018
	Nornicotine, %, by cyanogen bromide method	1.88	2.16	1.60	0.85

COMPARISON OF CYANOGEN BROMIDE AND OFFICIAL SILICOTUNGSTIC ACID METHODS

Composite samples (representing a large number of individual lots) of the four main types of tobacco used in cigaret manufacture were obtained and 5-gram aliquots submitted to steam-distillation by the official silicotungstic acid method (1). Aliquots of the distillates were analyzed for nicotine by the silicotungstic acid method and for nicotine and nornicotine by the cyanogen bromide method (first four columns of Table I).

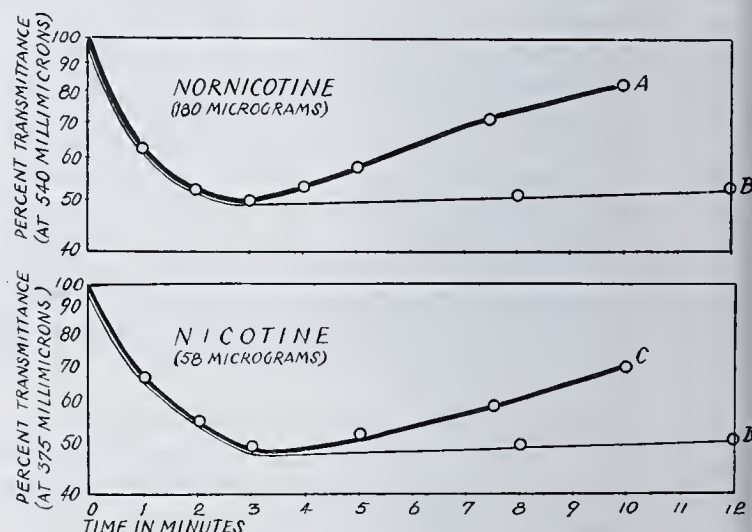


Figure 4. Development of Color

A and C, rate of development and fading of colors at 80° C. B and D, rate of fading of colors when reaction mixtures are cooled to room temperature immediately following 3 minutes at 80° C. All reactions carried out at pH 4.1, in presence of 0.4 gram of potassium dihydrogen phosphate.

Nornicotine is considerably less volatile with steam than is nicotine and the distillate as collected above may be expected to contain less than half of the nornicotine present in the tobacco. Two-gram samples of these same tobaccos were therefore submitted to the more drastic distillation described under "Preparation of Tobacco Distillates" for the cyanogen bromide method, which in the authors' experience gives quantitative recovery of nornicotine. Results of analyses by the silicotungstic acid and cyanogen bromide methods are shown in the last four columns of Table I.

Of tobacco of cigaret quality, Burley tobacco on an average seems to contain the greatest amount of nornicotine and Turkish tobacco the least. Individual lots of tobacco will not always follow this general rule.

On an average, the amount of nornicotine appearing in the distillate in the official silicotungstic acid method is sufficient



to cause a 5 to 8% error in the nicotine analyses (columns 1 and 3, Table I). However, the error in analysis of individual lots of tobacco will not always fall within these limits.

Table II shows the results of analysis by the official silicotungstic acid and the cyanogen bromide methods of four lots of high-nornicotine Maryland tobacco. These tobaccos well illustrate the fact that in certain strains nornicotine may be the predominant alkaloid. In such tobaccos the apparent nicotine content as determined by the official silicotungstic acid method may be greatly in error.

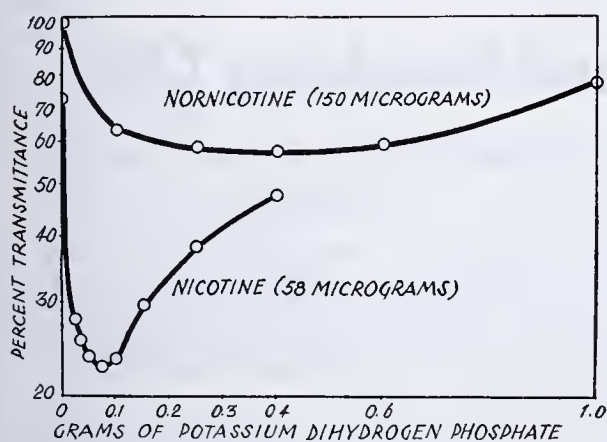


Figure 5. Effect of Potassium Dihydrogen Phosphate on Intensity of Color

Constant amounts of nornicotine and nicotine reacted with cyanogen bromide for 3 minutes at 80° C. All colors developed at pH 4.1. Transmittance measurements made at 540 millimicrons for nornicotine and 375 for nicotine.

**SPECIFICITY OF CYANOGEN BROMIDE METHOD.** Table III shows the relative intensities of colors developed by certain nicotine and pyridine derivatives under the conditions of the cyanogen bromide method. Nicotyrine and pyridine are steam-volatile substances known to be present in *Nicotiana glauca*. Anabasine is known to be present in certain other species of *Nicotiana*.

Table III. Color Development of Nicotine, Nornicotine, and Other Pyridine Derivatives

Substance	Nicotine Equivalent, %	Nornicotine Equivalent, %
Nicotine	100	0
Nornicotine	39	100
Nicotyrine <sup>a</sup>	14	0
Anabasine	22	101
Metanicotine	15	0
3-(1-Aminobutyl)pyridine	12	207
2-Aminonicotine	0	0
6-Aminonicotine	0	0
6(4-Aminobutoxy)-3,2'-nicotine <sup>a</sup>	0	0
Nicotinic acid amide	127	0
Nicotinic acid	130	0
$\beta$ -Picoline	0	0
$\alpha$ -Picoline	0	0
Pyridine	11	0

<sup>a</sup> Kindly furnished by Alfred Burger, University of Virginia, Charlottesville, Va.

Of this group, nornicotine, 3-(1-aminobutyl)pyridine, and anabasine are the only substances yielding a red color with cyanogen bromide. In none of these three is the nitrogen, of the group substituted in the beta position in the pyridine molecule, methylated. This, seemingly, is a necessary condition for the formation of a red color by nicotine derivatives when reacted with cyanogen bromide. Anabasine also yields a red color when reacted with cyanogen bromide in the absence of phosphate. This should make it possible to utilize the cyanogen bromide reaction to determine nornicotine and anabasine in mixtures.

Theoretically (12), pyridine derivatives involving substitution

on the alpha carbon should yield no color when reacted with cyanogen bromide. The authors' findings (Table III) are consistent with this and, in all probability,  $\alpha$ -nicotine and its derivatives would not interfere in the proposed method.

As seen in Table I, the sum of nicotine plus nornicotine as determined by the cyanogen bromide method is consistently slightly less than the total apparent nicotine as determined by silicotungstic acid precipitation. Unless this is due to some unrecognized error in the cyanogen bromide method, this may mean that some of the minor steam-volatile alkaloids of tobacco that form insoluble silicotungstates appear to a lesser degree in the proposed cyanogen bromide method. If so, these must be of the order of volatility of nicotine, since the more drastic steam-distillation did not increase this difference.

From the standpoint of specificity, then, the cyanogen bromide method, like the methods proposed by Markwood (10) and by Bowen and Barthel (4), essentially divides the tobacco alkaloids into those of the nicotine type and those of the nornicotine type.

Table III does not indicate the maximum color-producing potencies of pyridine and of its beta-substituted derivatives listed, other than nornicotine. Probably each has its individual optima of salt concentration, temperature and time of color development, pH, and characteristic wave length of minimum transmittance. The cyanogen bromide reaction with pyridine and its beta-substituted derivatives offers fertile possibilities for formulation of methods for the chemical determination of any individual member, even in the presence of other members, provided the present tendency to add an aromatic amine to the reaction is avoided.

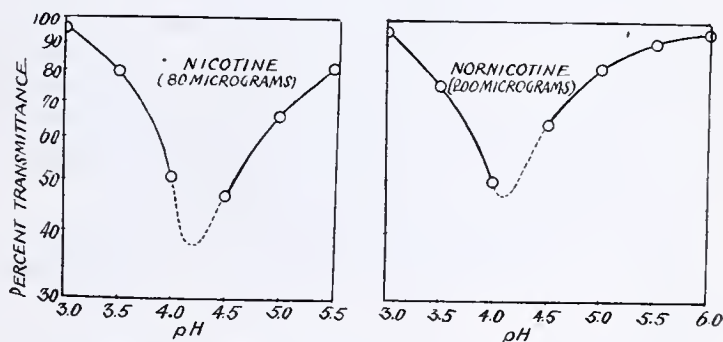


Figure 6. Effect of pH on Development of Color

Constant amounts of nicotine and nornicotine reacted with cyanogen bromide for 3 minutes at 80° C. in presence of 0.4 gram of potassium dihydrogen phosphate. Transmittance measurements made at 540 millimicrons for nornicotine and 375 for nicotine.

**NORNICOTINE TRANSFER INTO SMOKE.** But little data are available concerning the per cent transfer of nornicotine from tobacco to smoke. Wenusch and Maier (13) have stated that only a small amount of nornicotine is transferred into the smoke from material containing it. The authors have previously reported (6), from studies on a specimen of low-nicotine tobacco, a nornicotine transfer into smoke of less than 4%.

Specimen D (Table II) seemed ideal for further studies along this line, since it contained virtually no nicotine and a fairly high per cent of nornicotine. Twelve cigarettes made from this tobacco were smoked according to the procedure described by Bradford, Harlow, Harlan, and Hanmer (5). The resulting smoke solution, analyzed for nornicotine by the cyanogen bromide method, showed a 4.8% transfer of nornicotine from the tobacco into the smoke. This is less than one fourth of the transfer that has been found for nicotine (5).

#### ACKNOWLEDGMENT

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## Analysis of Bodied Drying and Semidrying Oils

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Operating details for determining the proportions and nature of those polymers in a heat-bodied vegetable oil resulting from self-addition of the fat acid portions of the oil are described. The method has been applied to the analysis of methyl esters bodied in the laboratory and of commercial oils.

BRADLEY (3, 4, 7) has emphasized the identity of drying phenomena with polymerization and the relationship between the functionality of oil molecules and their capacity to form convertible films. In particular, he points out (2) that one factor of importance in more completely understanding the drying mechanism of oils consists in the ascertainment of "the shape and size of the molecular aggregations at the sol-gel transition point". In addition to considerable amounts of unpolymerized fat acid glycerides, a heat-bodied oil consists of polyesters of polymeric fat acids with glycerol. A convenient method of determining the relative proportions and molecular size of these acids would be valuable not only for characterization of polymerized oils, but also for use in the preparation of condensation polymers from the polymeric fat acids.

The mode of origin and the chemical nature of the polymeric fat acids have been discussed by Bradley (5, 6, 7), Brod, France, and Evans (8), Kino (11, 12), and Ault (1) and will not be considered here.

The monomeric fat acid is readily separated from the mixture of polymeric fat acids by distillation of the methyl esters, but Bradley and Johnston (5) reported that the polymeric methyl esters were nonvolatile at 300° C. and 1 mm. in Claisen flasks. Kino (11) partially separated dimeric and trimeric methyl esters by solvent extraction. Bradley and Johnston (6) were able to isolate relatively pure dimers and trimers from polymerized dehydrated castor methyl esters by molecular distillation in a cyclic still. Likewise, Morse (13) fractionated a polymerized fish oil. While molecular distillation gives a good estimation of the proportions of monomeric, dimeric, and trimeric fat acids, experimental difficulties and time-consuming operations detract from the use of this method as a routine tool.

As a part of the general program of the Oil and Protein Division of the Northern Regional Research Laboratory concerned with the polymerization phenomenon of oils, the separation of polymeric fat acids was studied. As a result, it was found possible to achieve fractionation of polymeric fat acids, in the form of their methyl esters, by distillation at a pressure of 1 mm. or less in a specially designed short-path alembic flask. By this method, data of sufficient accuracy to be employed in the equations of Flory (9, 10) are obtainable, and the maximum extent of reaction of polymeric fat acids with various difunctional molecules is readily estimated. Furthermore, the characterization of bodied oils in terms of their dimeric and trimeric fat acid content can now be studied more conveniently.

#### APPARATUS

The apparatus used is shown in Figure 1. It may be constructed in various sizes. Capacities from 10 ml. to 5 liters have been used successfully at this laboratory, although more accurate results are obtained with the 1- to 2-liter sizes. The dimensions are not critical. Those shown in the figure are satisfactory for a flask of 1-liter capacity. Other sizes have proportional dimensions.

The flask is equipped with two side arms, A and B, for introduction of a thermometer and of a capillary tube through which an inert gas, usually carbon dioxide, is passed in order to prevent bumping and to provide an inert atmosphere. When flasks of 200-ml. or less capacity are used, bumping is controlled by packing the flask with glass wool; no side arm is then necessary for the capillary. A second thermometer, C, is placed in the neck of the alembic to obtain vapor temperatures. It is fitted with a splash baffle plate made by boring a hole in a Pyrex disk and attaching this to the thermometer with a small clip of Nichrome wire. The flask may be readily heated by use of an air bath or glass heating mantle.

The large side arm, D, leads to a small McCleod vacuum gage and thence to the pumping system. A good rotary vacuum pump is satisfactory for flask sizes up to 500 ml.; for larger sizes a mercury diffusion pump is necessary.

The arm, E, carries the distillate to a fraction cutter. If approximate results are desired, an ordinary "pig" carrying at least

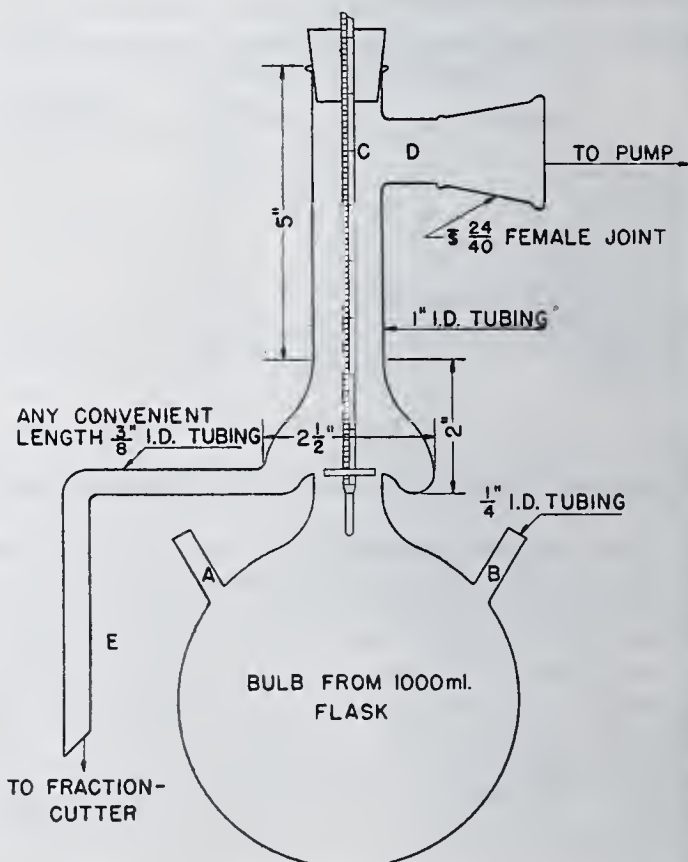


Figure 1. Apparatus



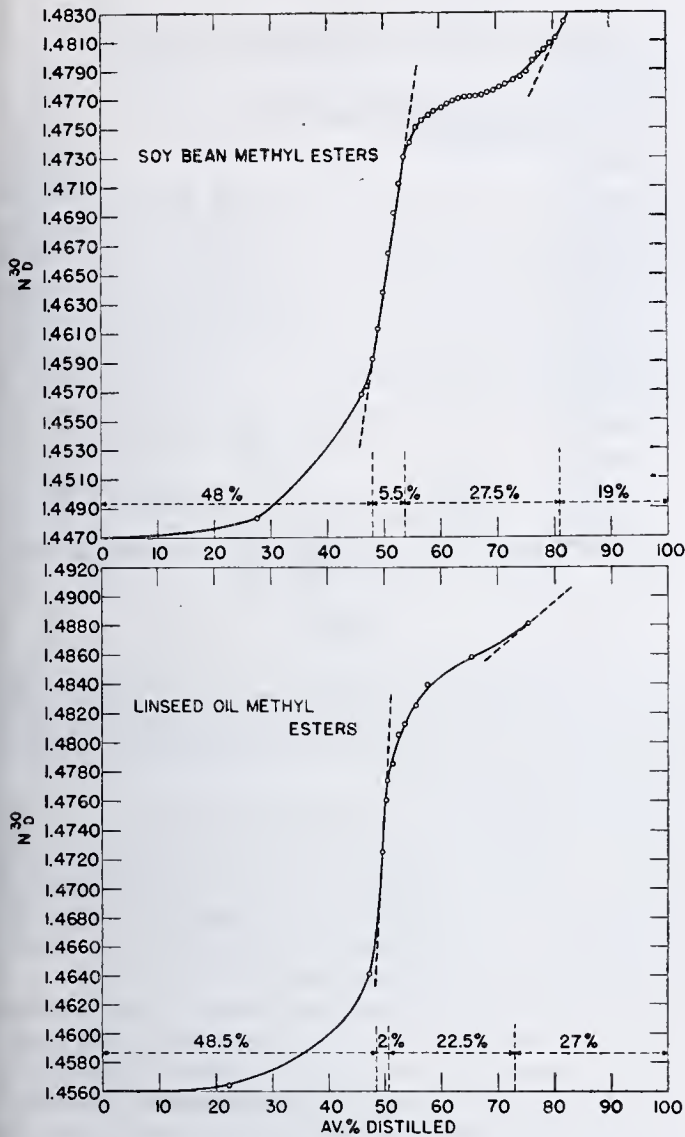


Figure 2

four test tubes or small flasks is adequate. For more accurate work or for large distillate volumes, a fraction cutter is necessary. Since the distillation must not be interrupted while changing receivers, an auxiliary pump is required.

PROCEDURE

**DISTILLATION.** The sample of bodied oil is converted to methyl esters either by transesterification in the usual manner

Table I. Refractive Indices of Methyl Esters		
Source of Methyl Ester	Before polymerization	After polymerization
Soybean oil	1.4538	1.4628
Linseed oil	1.4585	1.4711
Perilla oil	1.4621	1.4791
Tung oil	1.4880	1.4905
Linseed oil (commercial bodied)	....	1.4682

or by splitting followed by re-esterification. Unpolymerized esters are removed by distillation at 5 to 10 mm., in either ordinary apparatus or the alembic flask. If large amounts of monomer are present, it is more convenient to remove it in ordinary apparatus than to use an alembic flask of a size disproportionate to the amount of residual polymeric material to be distilled. The pot temperature during removal of monomer should not exceed 210° C. in order to avoid any possibility of polymerization.

The manner of conducting the remainder of the distillation depends upon the method of removing monomer. If an alembic flask is used, the pressure is slowly lowered to 1 mm. or less and distillation is continued; the material is not allowed to cool during this process. Small fractions of approximately equal weight are collected during the course of the distillation. When the pot temperature reaches 300° C., the distillation is stopped.

If removal of monomer is conducted separately, a sample of residual material is charged into an alembic flask of appropriate size, then degassed by warming while the pressure is slowly lowered to 1 mm. or less. When foaming subsides, distillation is begun and conducted as described above.

When a pig is used as fraction cutter, fractions are collected in

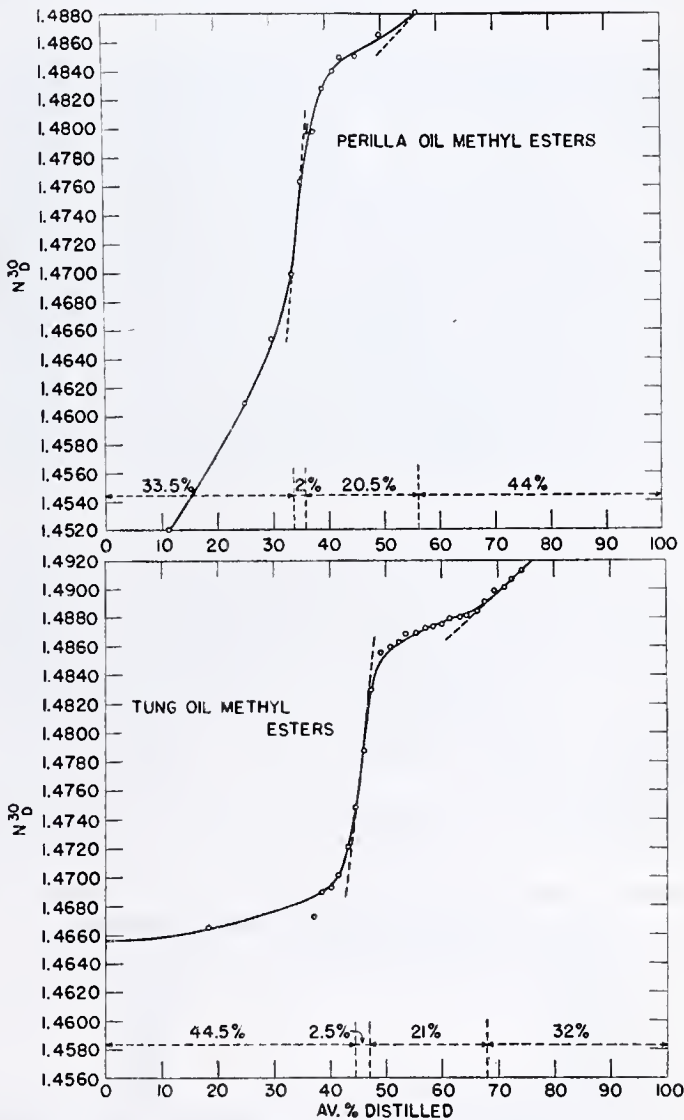


Figure 3

Table II. Analysis of Bodied Methyl Esters										
Fraction	Refractive Index Range (n <sub>D</sub> <sup>30</sup> ) for Various Fractions					Per Cent of Total				
	Soybean oil	Linseed oil	Perilla oil	Tung oil	Commercial bodied linseed	Soybean oil	Linseed oil	Perilla oil	Tung oil	Commercial bodied linseed
1. Monomer	Up to 1.4590	Up to 1.4676	Up to 1.4700	Up to 1.4732	Up to 1.4646	48.0	48.5	33.5	44.5	60.5
2. Intermediate	1.4590-1.4730	1.4676-1.4772	1.4700-1.4764	1.4732-1.4820	1.4646-1.4816	5.5	2.0	2.0	2.5	5.5
3. Dimer	1.4730-1.4812	1.4772-1.4874	1.4764-1.4880	1.4820-1.4890	1.4816-1.4890	27.5	22.5	20.5	21.0	16.5
4. Volatile higher polymers	1.4812-1.4924	1.4874-1.4880	.....	1.4890-1.4912	1.4890-1.4901	1.0	2.5	....	6.5	2.0
5. Residue	1.4869	1.4948	1.4970	1.4992	1.4975	18.0	24.5	44.0	25.5	15.5



the ranges up to 225° C., between 225° and 240° C., and between 240° and 265° C.

**ANALYSIS OF RESULTS.** The refractive index of each fraction collected is plotted against the total per cent distilled up to the mid-point of that fraction, and a smooth curve is drawn. This curve will indicate the presence of two fractions. The lower plateau corresponds to monomer and the higher plateau to dimer. The transition between these is designated as the intermediate fraction. [The intermediate fraction appears to consist of thermally cracked products or materials formed by recombination of cracked fragments (5).] The per cent of total represented by each fraction is readily determined from the graph, as shown in Figures 2, 3, and 4. A fraction collected between definitely established refractive indices will not necessarily correspond to monomer, intermediate, or dimer, unless all experimental variables are held constant. If distillation is carried out slowly with small flasks and a pig and the pressure is 1 mm., the temperature ranges suggested above may be considered to correspond to monomer, intermediate, and dimer, respectively. This procedure is not recommended for analytical work unless circumstances permit no alternative. However, it is valuable for rapid preparation of intermediate and dimer fractions of sufficient purity for many purposes.

#### EXPERIMENTAL

Distilled samples of soybean, linseed, perilla, and tung oil methyl esters were bodied without catalyst for 40 hours at 295° C., except in the case of tung oil which was bodied for 20 hours. (To illustrate the distillation technique, it is immaterial whether the oil or its methyl esters is bodied.) Comparative refractive indices of unbodied and bodied esters are given in Table I. Monomer was removed from the bodied esters, and the residues, amounting to approximately 1 kg. in each case, were submitted to distillation in alembic flasks of 2-liter capacity. A mercury vapor diffusion pump was necessary to secure the required pressure, which was about 0.005 mm. at the start of the distillation and increased slowly to about 0.01 to 0.03 mm. at the end. Pot temperatures ranged from 220° to 300° C. during the course of each distillation and vapor temperatures from 170° to 265° C. Small fractions, representing from 1 to 5% of the total, were collected and their refractive indices measured. These data were then graphed, taking account of the amount of monomer recovered in the initial distillation. The results are shown in Figures 2 and 3.

The composition of each bodied methyl ester is given in Table II, together with the refractive indices assigned to each fraction by inspection of the graphs. A smaller amount of nonvolatile residue than would be expected was obtained in the case of tung oil, since some polymerization occurred during the initial distillation of the unbodied esters. Molecular weights of the dimer fractions as determined cryoscopically in benzene are given in Table III.

Also included is the analysis of a sample of commercially bodied linseed oil of X viscosity (Gardner-Holt scale). This

Table III. Molecular Weights of Dimer Fractions

Source of Methyl Esters	Molecular Weight
Soybean oil	590
Linseed oil	633
Perilla oil	590
Tung oil	598
Corn oil	584
Commercial bodied linseed oil	600
(Theoretical)	588

Table IV. Distillation Data for Approximate Analysis of Bodied Oils

Oil	Fraction	B. P. Range, °C.	$n_D^{20}$	Weight, Grams	%
Bodied corn oil esters	Before distillation	.....	1.4767	59.0	..
	1	175-225	1.4656	8.3	14.0
	2	225-240	1.4704	10.2	17.3
	3	240-265	1.4760	25.9	44.0
	Residue	.....	1.4857	14.6	24.7
Commercial alkali-conjugated soybean oil esters	Before distillation	.....	....	73.3	..
	1	132-139	1.4565	28.2	45.0
	2	155-240	1.4611	25.4	36.3
	3	240-260	1.4795	7.5	10.7
	Residue	.....	....	5.6	8.0

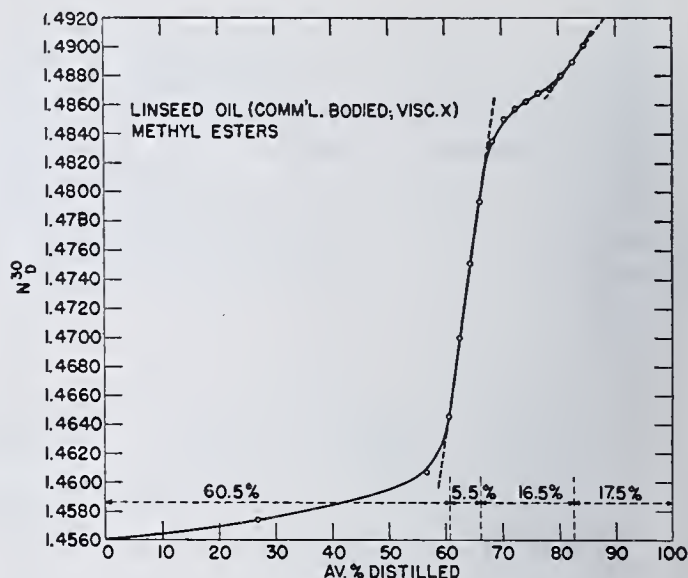


Figure 4

oil was transesterified with methyl alcohol using sodium methylate as a catalyst and then distilled in an alembic flask without previous removal of monomer. The distillation data are shown graphically in Figure 4.

In order to determine the closeness of fractionation and to serve as a check upon the accuracy of estimation of fraction size, a sample of distilled methyl esters of polymeric soybean fatty acids, collected in the refractive index range 1.4750 to 1.4800, was redistilled. It was found that only 6.4% of material of refractive index outside this range was present.

Illustrative of the results obtainable in small flasks with pig type fraction cutters are the data obtained by distillation of samples of methyl esters of polymeric corn fat acids and of methyl esters of commercial alkali-conjugated soybean fatty acids. A 59-gram sample of polymeric corn methyl esters, from which most of the monomer had previously been removed, was distilled in a 100-ml. alembic flask at a pressure of 0.5 to 1 mm. of mercury, secured with an ordinary rotary vacuum pump. The flask was packed with Pyrex glass wool to prevent bumping, and was provided with vapor and pot thermometers. The distillation data, given in Table IV, indicate the presence of 14% monomer, 17.3% intermediate, 44% dimer, and 24.7% residue consisting of trimer and higher polymers.

A sample of commercial alkali-conjugated soybean oil was saponified and re-esterified with methyl alcohol. Unpolymerized monomer was not removed prior to distillation in the alembic flask. Distillation data are shown in Table IV. The presence of 45% monomer, 36.3% intermediate, 10.7% dimer, and 8.0% trimer and higher polymers is indicated.

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# Determining Plasticizer Content of Cellulose Esters

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Plasticizer content is determined by the vacuum distillation of the plasticizer from the sample. Dry samples of 1 gram or less are placed in weighing bottles on the floor of a special vacuum still heated with Dowtherm. The sample is quickly converted to a film by action of a solvent and heating is continued for 1.5 hours. The loss in weight is due to plasticizer plus a slight decomposition of the cellulose ester. The latter is determined on a blank but is usually small enough and uniform enough that a fixed value may be assumed for it. Plasticizer content is given within about 0.3%.

THE determination of the plasticizer content of cellulose ester plastics has always been a slow operation, usually requiring large volumes of solvent and sometimes resulting in ambiguous values. The principal methods used have been (1) the extraction method, in which the sample is converted to a film, weighed, and extracted with ether, and either the extracted film or the extract is weighed, and (2) the precipitation method in which the weighed sample is dissolved in a small volume of solvent, the solution is poured into a large volume of nonsolvent, the precipitated cellulose ester is removed by filtration, and the plasticizer is recovered and weighed. While these methods, in the hands of a careful operator, can be used successfully, they are liable to serious error at several points. One source of trouble is the great tenacity with which cellulose esters cling to acetone and even to ether. Another is the obvious difficulty in removing the solvent quantitatively from the plasticizer without loss of the latter when many plasticizers have appreciable vapor pressure at the temperature required.

The method described in this paper consists of the removal of the plasticizer from a weighed sample of plastic by distillation under controlled conditions of temperature and pressure, and was developed as a result of an observation by W. O. Baker of these laboratories that additional plasticizer could be removed by vacuum distillation from a sample which had already supposedly been freed of plasticizers by ether extraction. Ryan and Watkins (1, 2) have used a method based on distillation of the plasticizer, but their sample (500 grams) was prohibitively large for an analytical method. The method developed here is fast and accurate, the technique is simple, it does not call for large quantities of solvents, and it requires only 1 gram or less of material.

## APPARATUS

The apparatus required, illustrated in Figure 1, can be made in any laboratory shop. The still itself consists of a steel double boiler, the top chamber of which has a 2.5-cm. (1-inch) flange or lip around the edge ground like a desiccator flange to fit the glass dome of a standard vacuum distillation apparatus (Corning Glass Co. No. 3480 EESHF). The apparatus is conveniently made of standard 0.6-cm. (0.25-inch) seamless steel tubing, 15 cm. (6 inches) in inside diameter, by welding on the flange, the partition, and the bottom. The partition, which constitutes the floor of the vacuum chamber, has a groove around the edge to collect any distillate which does not leave the chamber. The reflux column and vacuum line are, respectively, 0.5-inch and 0.375-inch iron pipe. Magnesia pipe lagging serves as the insulation.

After being welded in place the flange should be turned as smooth as possible on a lathe, and when the whole apparatus is finished the flange must be ground to a perfectly plane surface. This is very important, since the satisfactory operation of the apparatus depends on maintenance of a good vacuum in the vacuum chamber. When the joint is properly ground an ordinary laboratory vacuum pump should be able to reduce the pressure in the apparatus to less than 5 mm. with no sealing compound in

the joint. The temperature is held at 256° C. by boiling Dowtherm and the heat input is controlled so that the Dowtherm refluxes about halfway up the column. The exact point reached by the condensing vapor is easily determined by passing a damp cloth along the outside of the pipe. A glass plug in the top of the dome seals the hole and at the same time serves as a convenient handle. The other pieces of apparatus required are standard items and are clearly indicated by the diagram.

## PROCEDURE

Samples of approximately 1 gram in any form—i.e., film, powder, or lump—are weighed into tared wide-type Pyrex weighing bottles (50 × 30 mm.), which are placed, with covers off, in a vacuum desiccator over fresh phosphorus pentoxide. The pressure is reduced to 5 mm. and the samples are dried overnight. When the desiccator is to be opened dry air from a calcium chloride tower is admitted. The bottles with covers on are reweighed and the weight of the dried sample is thus obtained. To each sample are added 3 cc. of acetonyl-acetone (Eastman Kodak Co., boiling point about 193° C.) and the bottles, without covers, are placed in the apparatus which has previously been carefully cleaned. The flange is cleaned before each run with fine Carborundum paper held against a flat metal disk to assure a vacuum tight joint. The acetonyl-acetone is a powerful solvent for cellulose esters and in 3 or 4 minutes the sample dissolves.

As soon as the solvent begins to boil the glass dome is set in place, care being taken to see that it makes a good joint, and with the stopcock open the vacuum pump is started. The stopcock is slowly closed, the operator keeping his hand on it while watching the samples to see that they do not boil over or splatter. If the boiling is too vigorous the vacuum is released slightly till the boiling subsides. In this way the full vacuum is applied as rapidly as possible, the usual period being about 10 minutes from the time the samples were placed in the apparatus. As the solvent evaporates, the cellulose ester is left in a thin foamy layer from which the plasticizer can readily escape. As soon as the full vacuum is applied the operator seals the flange joint with a good sealing compound and the pressure in the system should then be 1 mm. or less. After exactly 1.5 hours from the time full vacuum was applied the stopcock is opened, the vacuum pump is shut off, the glass dome is removed, and the samples are lifted by means of tongs and quickly placed in a desiccator to cool. After half an hour the bottles with covers on are reweighed.

The dry samples should not be exposed to the air uncovered before weighing, since dry cellulose esters absorb water rapidly. The weighing bottles can be cleaned for re-use very conveniently after being soaked in acetone for a few hours. The loosely adhering ester is wiped off, and the bottles are rinsed in acetone, wiped dry with a cloth, and finally gently ignited and left to cool in a desiccator.

## DETERMINATION OF BLANK

The loss in weight of the samples put through the procedure described above represents plasticizer plus a certain amount of decomposition of the cellulose ester.

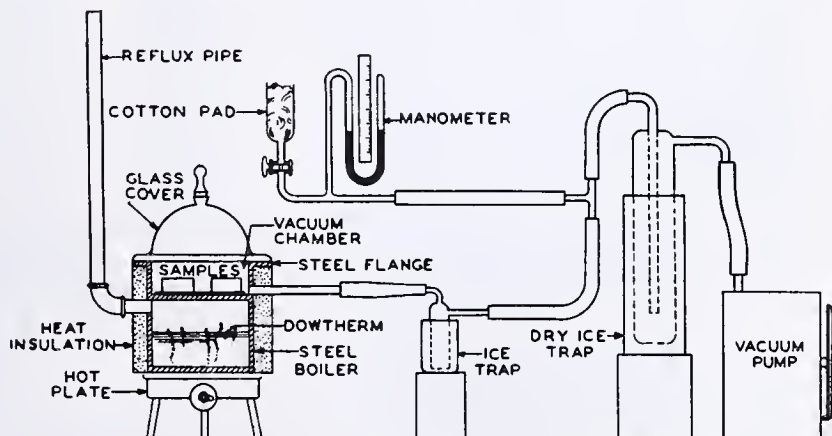


Figure 1. Vacuum Distillation Apparatus



Table I. Determination of Plasticizer

Cellulose Acetate- Butyrates	% Plasticizer		Cellulose Acetate- Butyrates	% Plasticizer	
	Lab. 1	Lab. 2		Lab. 1	Lab. 2
1	8.5	8.6	14	12.4	12.4
2	9.5	9.3	15	15.7	15.7
3	8.4	8.3	Cellulose acetates		
4	9.5	9.4			
5	8.5	8.7		26.1	25.9
6	8.6	8.7		26.2	26.2
7	8.6	8.4		26.1	26.0
8	8.6	8.5		25.2	25.3
9	8.5	8.5		23.0	25.3
10	8.5	8.6		26.1	26.1
11	8.8	8.7		24.9	25.0
12	8.7	8.5		25.5	25.5
13	8.8	8.7	24	25.2	25.0

In order to determine the latter, dry samples of unplasticized cellulose ester of the type to be analyzed are put through the standard procedure, an approximately normal amount of plasticizer being added along with the acetonyl-acetone to make the conditions comparable. The result can be calculated either on the plasticized or unplasticized weight of cellulose ester. The former is usually more convenient. For example, if samples under examination contain about 25% of plasticizer, for the blank, one takes 0.75 gram of unplasticized ester, adds about 0.25 gram of plasticizer, and determines total weight loss in per cent. The blank correction representing decomposition is:

$$\frac{\text{Weight loss of sample (not including plasticizer)} \times 100}{\text{Weight of sample plus plasticizer}}$$

This figure, which for cellulose acetate is usually about 0.3%, is directly applicable to plasticized samples and when subtracted from per cent total weight loss gives the correct plasticizer content. In the authors' experience, cellulose acetate-butyrate samples have a slightly greater initial loss in weight than the acetate, but the curve of the decomposition with time at 256° C. is much flatter after this initial loss than it is with acetates. Furthermore, acetate-butyrate usually contain considerably less plasticizer than acetates, and therefore the correction factor is

much more nearly the actual decomposition loss of the sample itself. The authors use a correction of 0.4% for acetate butyrate ranging from 0 to 15% in plasticizer content. For very exact work one can either make an absolute correction without regard to plasticizer, or determine the blank for the range of plasticizer content of the samples to be studied.

The method has been used in these laboratories on cellulose acetate samples varying in plasticizer content from 18 to 40% and on acetate-butyrate samples from 2 to 20% with excellent results. The plasticizers which these samples contained were diethyl phthalate, dibutyl phthalate, dibutyl sebacate, and butyl stearate, and no difficulty was encountered with volatilizing any of them. One would expect difficulty with the very high boiling materials such as dioxenyl cresyl phosphate and similar compounds. The method is particularly useful for routine control where large numbers of samples are to be run, since one operation over a period of several days can run in duplicate an average of six to eight samples per day. The method has also been used for following plasticizer distribution by analyzing single particles of molding powder or fractions of molding powder separated by flotation methods.

The reproducibility of the method is illustrated by Table I, results obtained on identical samples in two different laboratories with different stills. Each result is an average of duplicate runs.

The precision of the method is illustrated by the following analyses of samples, all taken from a single molded object and all analyses being run by the same operator.

Sample	% Plasticizer	Sample	% Plasticizer
1	25.16	4	24.94
2	24.95	5	24.99
3	25.01	6	25.07
		Av.	25.01

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## Vacuum-Jacketed Ground-Glass Joint for High-Vacuum Distillations at Elevated Temperatures

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**M**OST packed or specially prepared distilling columns are attached to the boiling flask by means of a standard taper ground-glass joint. High-vacuum distillations at elevated temperatures through these columns become difficult when the lubricant used on the tapered joint is extracted or rendered too fluid to be effective as a seal, owing to continued high temperatures. Prolonged heating distorts the joint and if special precautions are not observed, the inner and outer members become frozen together upon cooling. These undesirable features have been largely eliminated by a modified vacuum-jacketed joint, by means of which the joint is kept at a sufficiently low temperature to permit the use of ordinary high-vacuum lubricants. Good seals have been maintained in distilling with bath temperatures as high as 300° C.

The column is so constructed that the tapered joint is part of the vacuum jacket

surrounding the distilling column and is thus out of contact with the distilling vapors. The vacuum jacket is extended for a short distance below the joint with an enlargement which approaches the sides of the neck of the boiling flask and serves as a baffle. This minimizes heat of the joint and prevents extraction of lubricant from the joint by the reflux liquid.

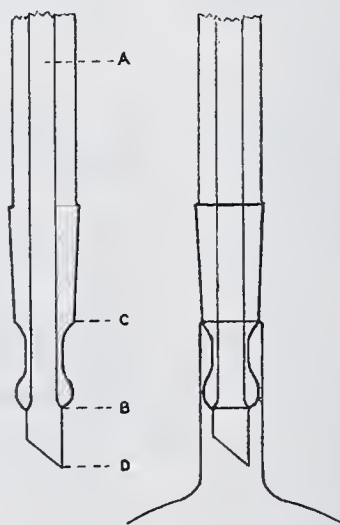


Figure 1

**APPARATUS.** Figure 1 shows the modified inner member alone and the inner member in position in the boiling flask. Tube is 8 mm. in diameter and is ring-sealed a 24/40 standard taper joint (Ace Glass Inc., Catalog No. 7640) 3 cm. below taper, C. The outer tube is enlarged immediately above the ring seal as illustrated. When this member is placed in position in the boiling flask the distance between the enlarged portion and the neck of the flask is about 1.5 mm. The drip joint, D, is about 2 cm. below the ring seal, B.



# Quantitative Determination of Crystalline Materials by X-Ray Diffraction

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A method is described for using x-ray diffraction patterns for chemical analysis of crystalline compounds. The line intensities, which are recorded by a suitable microphotometer, are corrected by means of simple graphical correction based upon an internal standard, to an arbitrary basis used for all comparisons. The accuracy of the method is limited by the microphotometer, grain size of the film, exposure variations, possibility of solid solution, etc., and will ordinarily be within 5% of the observed value. Mixtures of materials are readily analyzed from a single pattern, after standard patterns are taken to determine the characteristic constants. Data are given for several compounds. The method is independent of absorption corrections, sample shape effects, wave-length radiation used, etc.

MOST efforts to determine quantitatively the amounts of various constituents in mixtures, using x-ray diffraction patterns, have been concerned largely with efforts to correct for absorption, sample shape, and other quantities which involve considerable calculation. Because of such complications these methods have not found extensive application up to the present time, although considerable use has been made of line comparison methods (1, 2).

The present investigation shows a method of using an internal standard, which serves to give comparative line intensities, enables a direct and simple correction factor to be determined for absorption, sample shape, etc., and also permits use of the maxi-

<sup>1</sup> Present address, General Aniline and Film Corporation, Easton, Pa. Miss Martin carried through some of the experimental work in this paper as part of the laboratory course in x-ray diffraction at the University of Illinois.

mum intensity value of the interferences rather than integrated intensity. As a result, such determinations may be easily carried through with little calculation and with considerable speed.

The method, while not sufficiently accurate for many purposes, will serve in some cases, and in certain special applications will enable determinations where no previous methods of analysis could furnish the desired information. Such a case is illustrated in the present paper, the analysis of a rhyolite for quartz. Ordinary chemical analysis permits only determination of total silica, but the diffraction method, which is dependent only on the nature of the compounds present, is not so restricted.

## THEORY

The intensity of a diffraction line from a given powder sample will vary directly as the mass of the material in the volume irradiated, provided all other factors are considered constant. In case an internal standard is added to a known amount of sample, it becomes possible to determine the amount of any given constituent in terms of the mass of the added internal standard. A single diffraction pattern of a mixture of known composition must be obtained to determine the proper relationship between the inherent intensity values of the two diffraction lines selected for comparison.

The intensity of a Debye-Scherrer powder diffraction line, neglecting absorption and other effects which accompany the experimental measurement, is given by the relation (4)

$$I = I_0 \frac{N^2 e^4 \lambda^3 V (1 + \cos^2 2\theta)}{m^2 c^4 32 \pi r \sin^2 \theta \cos \theta} p F^2 \quad (1)$$

$I$  represents the diffracted intensity for a sample having  $N$  unit cells per cubic centimeter with a total irradiated volume  $V$ . The other symbols have their usual significance. The observed intensity of such a powder diffraction line is not completely expressed by the above equation, since corrections for sample shape (geometrical factors), absorption of the sample, etc., are not considered. If these corrections, which are all functions of the scattering angle  $2\theta$ , are all summed into a function  $A(d)$  (expressed in terms of the "interplanar spacing"  $d$  given by the Bragg law,  $\lambda = 2d \sin \theta$ ) we may write

$$I = K_0(d) A(d) V = K(d) A(d) C \quad (2)$$

where  $A(d)$  represents all corrections for the experimental conditions used in making the diffraction pattern, and  $K(d)$  all terms from Equation 1 except  $V$ , the volume of reflecting material in the irradiated volume of the sample.  $V$  is proportional to  $C$ , the per cent of the material present in the powder sample investigated.

Diffracted intensities for a pure compound in terms of the above equation would be

$$I_0 = K_0(d) A_0(d) C_0 \quad (3)$$

where  $C_0 = 100\%$ ,  $A_0(d)$  represents the  $A(d)$  curve for this particular material and the particular manner in which the pattern is taken, and  $K_0(d)$  involves

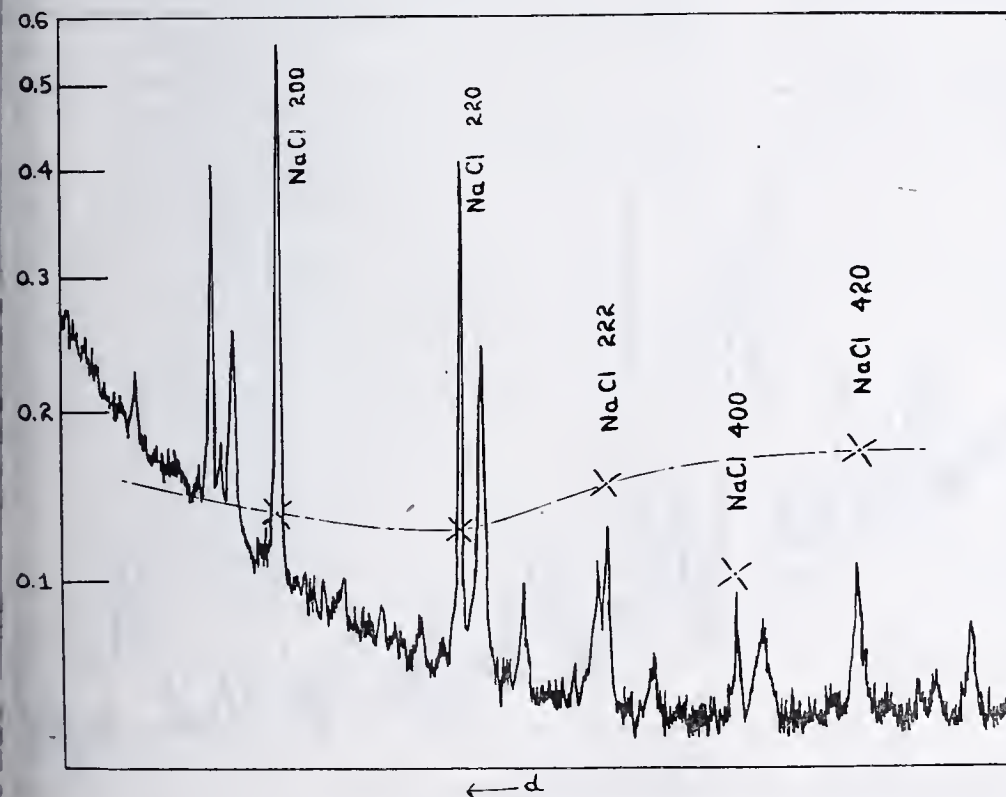


Figure 1.  $I_0/I_s$  Curve for Pattern of Mixture

Composition: 40% NaCl, 30%  $\text{CaF}_2$ , 30% quartz (pattern 5, Table II). Crosses indicate value (density scale  $\times 10$ ) for each diffraction line. NaCl 400 line gives a ratio not in agreement with the rest, probably because of superimposed interference from one of the other constituents at this point.



the quantities indicated in Equation 1 except  $V$ . If the sample contained only a portion of this particular standard material, the diffraction intensities would be

$$I_s = K_0(d) C_s A(d) \tag{4}$$

$I_s$  represents the intensities of the various diffraction lines due to constituent  $s$ ,  $C_s$  the percentage of the standard material,  $s$ , present, and  $A(d)$ , since it is independent of everything but quantities dependent upon the size, absorption, and shape of the sample, must be the same for all other materials in the mixture. This permits the evaluation for the observed intensity of diffraction for a given constituent,  $c$ , which is to be determined.

$$I_c = C_c K_c A(d) = \frac{C_c K_c I_s}{K_0 C_s}$$

but 
$$K_0 = \frac{I_0}{C_0 A_0(d)}$$

so that 
$$C_c = \frac{I_0 I_c C_s}{I_s K_c C_0 A_0(d)} \tag{5}$$

We may define a new function,

$$k_c(d) = 1/K_c C_0 A_0(d)$$

where  $k_c(d)$  assumes values at various  $d$  positions fixed by the scattering properties of the material to be determined and the properties of the standard material used as an internal standard. Substitution of this last equation in 5 leads to

$$C_c = C_s I_c (I_0/I_s) k_c(d) \tag{6}$$

$k_c(d)$  is a constant for any given diffraction line in a pattern, provided a given internal standard,  $s$ , is used, and will have different values for the different lines in the pattern. The set of  $k_c(d)$  values from pattern to pattern, however, is identical.

$C_s$  is the percentage of the desired constituent in the mixture (including the internal standard material as an ingredient of the mixture).

$C_0$  is the percentage of internal standard.

$(I_0/I_s)$  represents the ratio of the intensity of a given standard line from the reference pattern of the compound used as internal standard, to the same diffraction line from the internal standard in the mixture. This value must correspond to the  $d$  value of the line indicated by  $K_c(d)$ . Therefore the ratio  $(I_0/I_s)$  is determined for every line due to the internal standard on the microphotometer curve, and plotted against  $d$  values. This curve permits interpolation and extrapolation to determine values of the ratio for the particular  $d$  values of interest.

$I_s$  is the intensity of the diffraction line used for examination.  $I_c(I_0/I_s)$  may be regarded as the corrected value of this line.

Usually it is desired to express percentages of constituents as they existed in the original mixture before any addition of internal standard is made. Then Equation 6 may be written

$$C_c \text{ (in original sample) } = \frac{I_c (I_0/I_s) k_c(d) s}{w} 100 \tag{7}$$

where  $s$  is weight of internal standard added to  $w$  grams of sample. The other terms have the same significance as indicated in Equation 6.

With the above expressions it is a simple matter to correct the microphotometer curve of the ordinary powder pattern for computations, since it is only necessary to determine the correction function,  $I_0/I_s$ , from the diffraction lines of the internal standard, plot the curve, and select the proper

value for the specific interference to be used for the analysis; it must be remembered, however, that the constant  $k_c(d)$  for a given diffraction line of the material to be estimated, and other diffraction lines even of the same pattern would necessarily involve other constant values.

### APPLICATIONS

The above treatment has been demonstrated for the circular camera type of pattern, but the same result is obtained for the use of the flat cassette method and the resulting powder halos; even the  $k_c(d)$  constants will have the same values. The correction curve,  $I_0/I_s$ , however, will show much greater slope if the standard reference pattern is essentially different in type from that used in the analysis (with consequent loss in accuracy). It is not necessary to specify radiation for the values of the constants since they are independent of wave length. Radiation source, however, should be well filtered to permit selection of a smooth background in estimating intensity values. The diffraction cameras should be of such size that good resolution of the diffracted lines is obtained, in order to avoid overlapping of interferences and also to help obtain smooth continuous background values. A film with reasonably small grain size is preferred for the same reason.

The sensitivity of the diffraction method of analysis varies considerably as a function of the specific materials concerned. Generally we may assume that about 1% would be the limit detectable in a mixture. In some cases—for example, the determination of platinum in platinized aerogel—quantities extremely minute may be determined, while in other cases, such as the determination of silica in lead oxide, quantities considerably larger

Table I. Sodium Chloride Pattern Used as Reference Internal Standard

Line No.	hkl	d, Å.	Relative Density
1	1 1 1	3.25	...
2	2 0 0	2.81	0.626
3	2 2 0	1.99	0.465
4	2 2 2	1.62	0.144
5	4 0 0	1.41	0.067
6	4 2 0	1.26	0.140

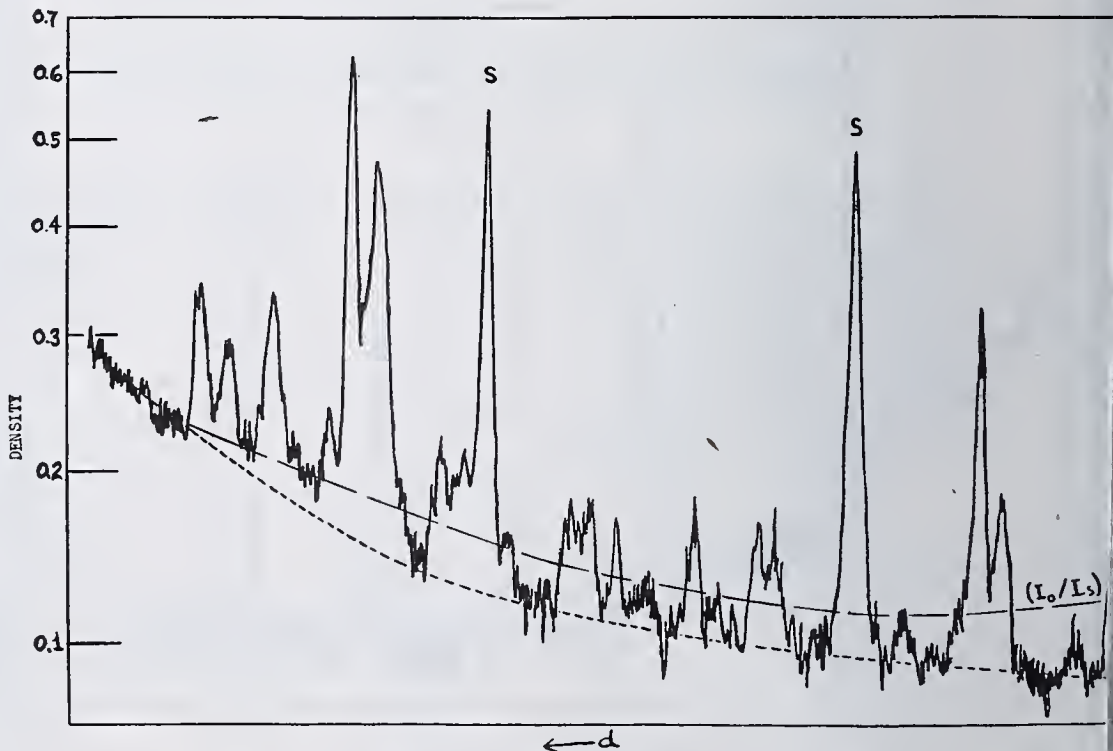


Figure 2. Microphotometer Tracing for First Sample in Table IV  
14.85% NaCl internal standard. Compare with Figure 3, same sample with 19.69% NaCl internal standard. Conditions of exposure varied over as wide a range as feasible in exposure. Internal standard lines (NaCl 200 and 220) indicated by S



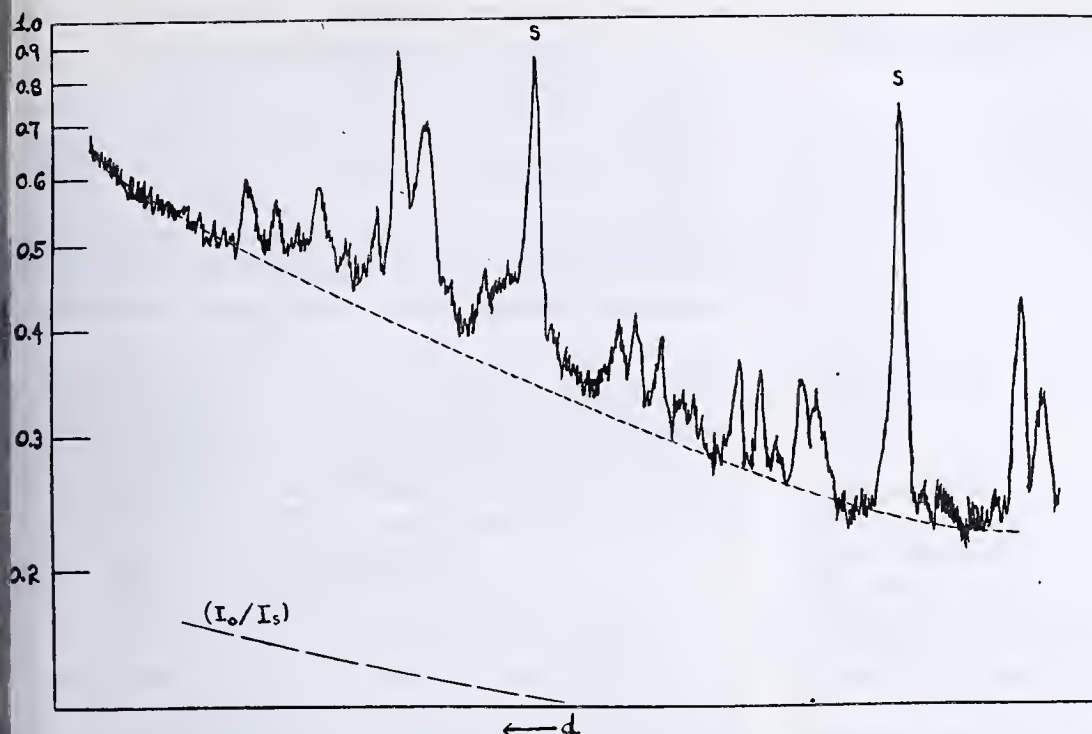


Figure 3. Microphotometer Tracing of Pattern for Rhyolite  
19.69% internal standard (see Table IV)

an 1% would be required. The method permits evaluation within the sensitivity of the photographic method; the use of the microphotometer and film automatically restricts the accuracy of the method to about 5%, and where the amount of internal standard does not compare favorably with the amount of the material being determined the error may become somewhat greater. The method is adaptable only for crystalline materials, and when colloidal materials or solid solution phenomena are present the routine analysis as presented in this paper would have to be considerably modified, and the results would not be so satisfactory. It is unnecessary to use the integrated intensities (area under the intensity-diffraction angle curve), since the experimental correction function,  $I_0/I_s$ , has the property of making the required correction.

A modification of the method which enables determinations on a micro scale has been developed and will shortly be published. This particular method permits analysis of alkaloids and other suitable materials in total quantities present of the order of 0.00001 gram.

The method could be made even more suitable and accurate by using the focusing back-reflection method with its great resolution and almost linear correction curve. The authors have not done this here, since it was considered advisable to use that type of pattern which is directly suitable for qualitative identification according to the Hanawalt (3) method.

With slight changes the method permits evaluation, within the sensitivity of the photographic method, of the absolute intensity of powder diffraction lines (except for the corrections brought out by the Debye-Waller temperature factor, etc.) and can be used to obtain Fourier or other data used in crystal structure determinations.

#### EXPERIMENTAL

All patterns were made using the wedge method with copper K $\alpha$  radiation filtered through 0.025 mm. (0.001-inch) nickel foil. Exposures were from 2 to 4 hours, using a Phillips Metallix diffraction x-ray tube operated at 28 kilovolts and 20 milliamperes and a camera with radius of 6.4 cm. The pattern of pure sodium chloride, which was used as a reference internal standard for these experiments, is listed in Table I with density values recorded by a Leeds & Northrup microphotometer.

Other materials are probably superior to sodium chloride for such an internal standard, since the sodium chloride pattern has comparatively few lines and the innermost usable line is rather far removed from the center of the pattern, requiring extrapolation of the  $I_0/I_s$  curve for many materials. It is recommended that the constant values to be used be determined previous to analysis, using the same lot of standard material (sodium chloride or other standard) which is to be employed in the analysis, in order to obviate errors which might arise through use of various lots of chemicals. Such an error would usually be small.

The  $I_0/I_s$  curves were plotted directly on the microphotometer record, using the density value scale multiplied by ten. The logarithmic density scale tends to flatten out the correction curve

and makes it simple to draw a continuous curve through the points obtained from the diffraction lines of the internal standard. The density values for the five interferences obtained from the sodium chloride are divided into those values given in the reference pattern in Table I to obtain correction factors for the positions corresponding to the particular interplanar spacings listed. These points are located on the microphotometer paper (see Figure 1) and a smooth curve,  $I_0/I_s$ , is drawn through them. This  $I_0/I_s$  curve then gives the proper correction value for every position on the pattern, and each interference to be used for calculation is corrected by multiplying its observed density value by the correction factor indicated. The microphotometer records and correction curves for a known mixture are shown in Figure 1.

A series of known mixtures containing quartz is shown in Table II with a comparison of observed and known percentages. The sodium chloride was added in known amount, and the percentage of sodium chloride in the final sample used is indicated.

Table II. Quartz in Mixtures Containing the Internal Standard (See Equation 6)<sup>a</sup>

Pattern	NaCl %	Known CaF <sub>2</sub> %	Mixture MgO %	SiO <sub>2</sub> %	$I_e(I_0/I_s)$ Quartz	Quartz Determined %
1	45	..	50	5	$0.045 \times 1.27$	4.95
2	45	..	50	5	$0.095 \times 0.65$	5.34
3	50	..	35	15	$0.178 \times 0.90$	15.4
4	50	..	35	15	$0.224 \times 0.69$	14.8
5	40	30	..	30	$0.277 \times 1.35$	28.7

<sup>a</sup> Percentage determined in mixture as given.

Table III. Characteristic Constants for Analysis of Minerals against a Sodium Chloride Internal Standard<sup>a</sup>

Mineral	$d$ , Å.	$K_c(d)$	Formula
Cristobalite	3.13	32.0	SiO <sub>2</sub>
Fluorite	3.16	3.93	CaF <sub>2</sub>
	1.65	8.71	CaF <sub>2</sub>
Quartz	3.35	1.92	SiO <sub>2</sub>
Tridymite	4.08	8.40	SiO <sub>2</sub>

<sup>a</sup> Determination of tridymite is not satisfactory, since the sodium chloride standard has no lines in the immediate vicinity of the three most important tridymite lines, requiring extrapolation over a rather considerable range, with a larger consequent error than is found in more favorable cases. A standard material other than sodium chloride, chosen so that numerous lines occur in the larger spacings, would obviate this difficulty, although the diffraction camera should provide suitable resolution.



Table III lists the characteristic constants for the analysis of several minerals against the given sodium chloride internal standard.

One great advantage of the present method of analysis over the usual chemical and physical methods employed for quantitative determination is inherent in the fact that the diffraction method is suitable for any crystalline material, and the patterns obtained are dependent upon the actual structure rather than the chemical composition. It is unnecessary to destroy a compound of interest by solution in solvents as a preliminary step in analysis—i.e. analysis of hydrates, polymorphic materials, etc. The method is not indicated for noncrystalline materials such as liquids and glasses, and must be used with caution in the presence of possible solid solutions and of materials with particle size in the colloidal range.

Table IV (and Figures 2 and 3) illustrate the use of the method in analysis of a rhyolite from the extrusion at Nathrop, Chaffee Co., Colo. The material contains quartz, tridymite, hyalite, cristobalite, spessartite (garnet), hematite, and topaz. The analysis was carried through for quartz and tridymite. The agreement of tridymite values is probably fortuitous, since an extrapolated correction value over a rather considerable range was used.

The present method reduces the number of synthetic specimens which must be employed to a single sample; it is, of course, al-

Table IV. Partial Analysis<sup>a</sup> of Rhyolite

NaCl %	$I_c(I_0/I_s)$ Quartz	$I_c(I_0/I_s)$ Tridymite	Quartz %	Tridymite %
14.85	$0.43 \times 1.87$	$0.08 \times 2.18$	26.8	25.5
19.69	$0.42 \times 1.42$	$0.08 \times 1.55$	28.1	25.4
			Av. 27.4	25.5

<sup>a</sup> Cristobalite content was not determined, since the intensity of the line was too faint to permit satisfactory measurement on the patterns obtained. Percentages are expressed for rhyolite before admixture of internal standard.

ways advisable to make up a synthetic sample duplicating the results of the analysis after the analysis is completed, at least until the general use of the method has been thoroughly tested for any particular analysis. This pattern could be used to refine the accuracy of the determination.

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## Determination of Total and Combined Sulfur in Butyl Rubber

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A procedure is described for determining total and combined sulfur in Butyl rubber vulcanizates. Methyl ethyl ketone has been found to be a satisfactory and inexpensive extraction solvent. The total sulfur in the vulcanizate and the combined sulfur remaining after extraction are determined as barium sulfate, following combustion of the samples in the Braun-Shell sulfur apparatus and conversion of the resulting sulfur oxides to sulfate by means of an alkaline sodium hypobromite solution. Extractable sulfur may be determined by difference.

IN THE course of certain polymer studies in this laboratory, it became necessary to determine the amounts of total and combined sulfur in Butyl rubber vulcanizates. No previously published method of analysis was available for this class of synthetic rubbers. It seemed worth while to disclose the analytical procedure described below, because it may have a wider possible field of application than that for which it was originally developed. Some of the problems that may be studied with the aid of this method are rate of vulcanization, behavior of various accelerators, sulfur blooming, and factory control.

The numerous methods that have been devised for determining total and combined sulfur in natural rubber compositions are adequately described, or referred to, elsewhere (2, 3, 6). A commonly used procedure consists in analyzing the composition for total sulfur by oxidation of the sulfur to sulfate with such reagents as nitric or perchloric acid, followed by determination of the sulfate in the usual manner by precipitation as barium sulfate. Free sulfur is considered to be completely extractable from the vulcanizate, exhaustive treatment with acetone being employed almost universally for this purpose. The sulfur in the acetone extract is commonly determined as barium sulfate, after oxidation with a nitric acid-bromine mixture. The difference between the total and extractable sulfur values is regarded as chemically combined sulfur. No discussion need be given here of the familiar complications sometimes introduced by the presence of inorganic sulfides and sulfates, some accelerators, and various compounding ingredients that contain sulfur.

Early in this work it was found that the procedure described for natural rubber could not be applied successfully to Butyl rubber. The principal reasons for the difference in behavior appeared to be twofold: the Butyl rubber compositions are less permeable to acetone, and their stability toward oxidizing agents exceeds greatly that of natural rubber. Neither the vulcanizates nor the acetone-extractable materials (which contain small percentages of low-molecular components of the polymer) could be readily oxidized, even after protracted treatment with the oxidizing solutions. Furthermore, acetone proved to be a very poor agent for removing extractable sulfur under the conditions employed in this work. It was found, however, that the latex could be completely extracted within 8 hours by means of methyl ethyl ketone. This solvent appears to swell the Butyl vulcanizates sufficiently well to hasten sulfur diffusion very markedly and its use does not result in the excessive oxidative depolymerization reported by Cheyney (1) for natural rubber. While it is conceivable that the behavior of acetone might be satisfactory in the method of hot extraction recommended for natural rubber by the A.S.T.M. instead of standard Soxhlet extraction as used in this work, it is believed that methyl ethyl ketone would also prove to be a superior extraction agent, although further experiments would be necessary to verify this point. It was also found that the total sulfur in the original vulcanizate, as well as the combined sulfur remaining after extraction with the ketone, could be readily determined as barium sulfate by burning the sample in a Braun-Shell sulfur apparatus (Braun Corporation, Los Angeles, Calif.), the sulfur oxides formed then being converted to sulfate by absorption in alkaline sodium hypobromite solution. Extractable sulfur is, of course, given by the difference between the total and the combined sulfur values.

In view of the ease with which these determinations can be carried out, the excellent results obtained, and the low cost of methyl ethyl ketone, it was considered unnecessary to study the applicability of other ketones, although the use of higher ketones might enable one to reduce still further the time required for complete extraction.



## APPARATUS

The air-purifying train is similar to that described by Zahn (8), and is composed of an air filter, furnace, cooler, gas-washing bottles, spray trap, manifold, and flowmeter. The air filter is inserted directly in the compressed air line. The air passes from this device into an electrically heated stainless steel tube partly filled with quartz chips and maintained at approximately 800° C. This leads to a water-jacketed copper coil cooling tube, from which the gas issues into a washing bottle containing 2% alkaline sodium hypobromite solution, followed by a similar bottle containing 2% sodium hydroxide. The purified gas then passes through a spray trap into a constant-pressure manifold, each outlet of which is equipped with a calibrated flowmeter. Constant pressure is maintained on the manifold by means of a mercury-water seal equivalent to 48 mm. of mercury. The purified air then passes directly into the combustion tubes of a Braun-Shell sulfur apparatus (Figure 1). The air-purifying train described above can doubtless be modified and perhaps simplified without any serious sacrifice in efficiency, and thereby be adapted to more modest available equipment.

The Soxhlet extraction apparatus consists of the standard, all-glass type, of a size sufficiently large to hold a 34 × 100 mm. Erlenmeyer thimble (RA98, coarse; A. H. Thomas Co.).

## REAGENTS AND SOLUTIONS

The acetone and methyl ethyl ketone are of commercial grade, dried with, and distilled from, anhydrous potassium carbonate prior to use. The alkaline sodium hypobromite solution is prepared by dissolving 112 grams of bromine and an equal weight of sodium hydroxide in 2600 ml. of distilled water, and the resulting stock solution is diluted for use with 3 volumes of distilled water. Except for the ketones, all chemicals used in this work are of reagent grade, and all solutions are prepared with distilled water.

## PROCEDURE

About 0.5 gram of the rubber sample is carefully weighed and cut into 2- to 3-mm. cubes. In this range of subdivision the rate of extraction of the material is not critical. The comminuted sample is placed in the thimble of the Soxhlet apparatus and extracted at a siphoning rate of approximately 5 minutes. When unvulcanized samples are used, the cubes cohere after a short period of extraction. This is successfully prevented by mixing the material with a small amount of 20- to 30-mesh Ottawa sand that has previously been extracted for 24 hours with methyl ethyl ketone. No cohesion of particles is experienced with any vulcanized samples. The extracted material is dried in an air oven at 100° C. in order to remove the ketone remaining in the sample. This drying step can probably be eliminated from a routine procedure.

The sample is then transferred to a porcelain combustion boat (Coors No. 2) and placed in the sulfur apparatus. Purified air is passed through the apparatus at a rate of 2 to 3 liters per minute, and the sample is burned with a Bunsen burner. A short subsequent heating with a Méker burner suffices to give a colorless ash, the entire burning operation being completed within 10 to 15 minutes. After passage of the decomposition products through the furnace zone of the apparatus, the resulting sulfur oxides are absorbed in 50 ml. of alkaline sodium hypobromite solution. The absorber liquid is then washed into a 400-ml. beaker, 25 ml. of hydrochloric acid are added, and the excess bromine is removed by boiling. The solution is diluted to about 250 ml. with distilled water, brought to boiling, 10 ml. of 10% barium chloride solution are added, and the precipitated barium sulfate is digested overnight on a steam bath. The precipitate is filtered, washed, dried, and weighed in the customary manner. For de-

Table I. Properties of Crude Butyl Rubbers

Polymer	Intrinsic Viscosity in Diisobutylene at 20° C. (4)	Molecular Weight, Viscosity Average (4)	Unsaturation, Mole % (7)
Butyl 1	1.25	370,000	0.51
Butyl 2	1.28	385,000	0.71

Table II. Extraction of Sulfur from an Unvulcanized Mixture

Time of Extraction Hours	Total S %	S ex- tracted %	Acetone		Methyl Ethyl Ketone		
			S Re- main- ing in sample %	Total ma- terial ex- tracted %	S ex- tracted %	S re- main- ing in sample %	Total ma- terial ex- tracted %
5	1.41	0.69	0.72	1.4	1.36	0.05	2.9
8	1.41	1.00	0.41	1.4	1.39	0.02	3.0
16	1.41	1.16	0.25	2.1	1.39	0.02	3.2
30	1.41	1.23	0.18	2.1	1.39	0.02	3.4
50	1.41	1.29	0.12	2.1	1.39	0.02	3.2
72	1.41	1.37	0.04	2.5	1.38	0.03	3.8

termination of total sulfur, the same procedure is employed, the extraction step being omitted.

With samples containing very small percentages of sulfur, or with unvulcanized compositions from which almost all of the sulfur has been removed by extraction, the barium sulfate is determined turbidimetrically by the method described by Zahn (8).

## EXPERIMENTAL RESULTS

A blank run was made for the purpose of determining the sulfur contributed by the reagents and apparatus. The amount of purified air passed through the apparatus was approximately equal to that used in the subsequent rubber analyses. A sulfur blank of 0.005% was obtained.

Two samples of Butyl rubber were used in preparing the compositions discussed in this paper. In Table I are recorded some characteristic properties of these two materials. Blank determinations carried out with samples of these two polymers gave sulfur values of 0.010% for Butyl 1 and 0.008% for Butyl 2, corrected for the reagent blank.

In order to determine the relative efficiencies of extraction by acetone and methyl ethyl ketone, a set of experiments was carried out with a milled, unvulcanized mixture composed of Butyl 1 100 parts, carbon black 7.0 parts, and sulfur 1.5 parts. Assuming no losses of moisture or of any of the components during mill mixing, the calculated sulfur content of the mixture was 1.39%. Analysis by the method under discussion gave values of 1.40, 1.42, and 1.41. The comparative behavior of the two solvents may be judged by the data given in Table II; acetone is not capable of removing the sulfur completely within 72 hours, whereas methyl ethyl ketone gives constant values within 8 hours. The small percentage of sulfur remaining in the sample after exhaustive extraction with the latter solvent is believed to be chemically combined as the result of milling, although the possibility of some

combination occurring during the extraction process must be recognized. Evidence for this view is presented below, and independent data supporting this conclusion have been obtained (5) in studies of the viscosities of similar milled mixtures. The superior swelling characteristics of the higher ketone are reflected in the data in Table II showing the total amount of material extracted, these values with methyl ethyl ketone being somewhat larger than the corresponding values with acetone. No significant amount of degradation occurred during extraction, the total amount of extracted material showing only a slight upward trend with extraction time.

Analytical data of a corresponding nature are presented in Table III for a routine mixture based on the recipe Butyl 1 100 parts, zinc oxide 2.0 parts, carbon black 7.0 parts, sulfur 1.5 parts,

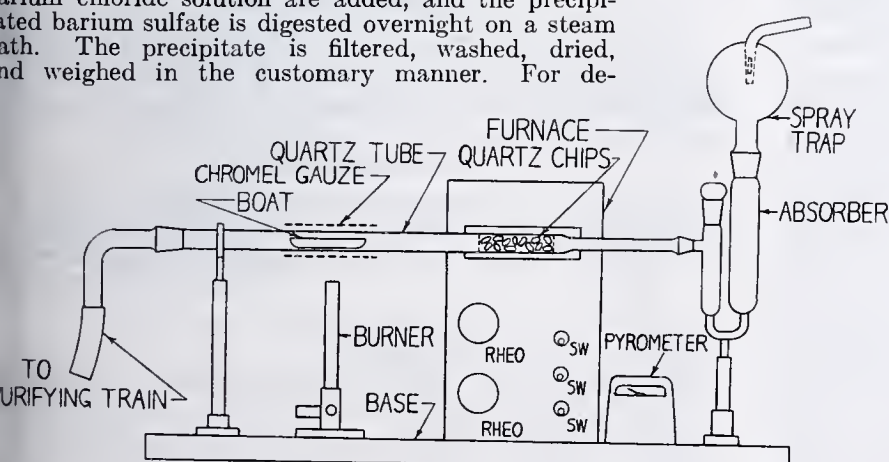


Figure 1. Diagram of Braun-Shell Sulfur Apparatus



Table III. Extraction of Sulfur from a Vulcanized Mixture

Time of Extraction Hours	Acetone				Methyl Ethyl Ketone			
	Total S %	S ex- tracted %	S re- maining in sample %	S in ash %	Material ex- tracted %	S ex- tracted %	S re- maining in sample %	S in ash %
5	1.70	0.76	0.94	0.012	1.1	1.10	0.60	0.014
8	1.70	0.81	0.89	0.008	1.7	1.16	0.54	0.010
16	1.70	0.96	0.74	0.014	1.8	1.15	0.55	0.014
72	1.70	1.11	0.59	0.016	2.4	1.16	0.54	0.014

Table IV. Extractability of Sulfur and Accelerators from Unvulcanized Mixtures with Methyl Ethyl Ketone

Composition	Time of Extraction Hours	Sulfur Remaining in Sample	
		Butyl 1 %	Butyl 2 %
Butyl 100}	8	0.05	0.06
Sulfur 3}	16	0.05	0.06
Butyl 100}	8	0.03	0.02
Tetramethylthiuram disulfide 1}	16	0.03	0.02
Butyl 100}	8	0.03	0.03
Mercaptobenzothiazole 1}	16	0.03	0.02
Butyl 100}	8	0.05	0.05
Carbon black 50}	16	0.05	0.05
Sulfur 1.5}			
Butyl 100}	8	0.06	0.07
Carbon black 50}	16	0.07	0.07
Tetramethylthiuram disulfide 1}			
Butyl 100}	8	0.06	0.07
Carbon black 50}	16	0.06	0.07
Mercaptobenzothiazole 1}			

and tetramethylthiuram disulfide 1.0 part: vulcanized for 60 minutes at 152° C. The total sulfur values for this composition were found to be 1.71, 1.69, 1.70%. (The calculated sulfur content, 1.82, is probably incorrect because of errors in compounding or losses during milling.) Table III shows that the extractable sulfur in the vulcanizate was not entirely removed after 72 hours of acetone extraction; on the other hand, constant values were again obtained within 8 hours with methyl ethyl ketone. The ash analyses show that roughly 0.01% of sulfur remained in the ash. It will be seen from Table VI that approximately the same percentage of sulfur was found in the ash of a composition containing 5.0 parts of zinc oxide.

In order to determine whether certain common accelerators are completely extracted under the above conditions, experiments were carried out with various unvulcanized mixtures, the compositions of which are given in Table IV. Parallel results are recorded for mixtures that had been prepared with the two polymers described in Table I. Table IV shows that extraction for 8 hours with methyl ethyl ketone is sufficient to remove virtually all of the sulfur, tetramethylthiuram disulfide, and mercaptobenzothiazole present in the compositions; furthermore, the presence of 50 parts of carbon black does not cause interference. The small percentages of residual sulfur shown in Table IV may be attributed to chemical combination during the milling procedure. The values for the mixtures containing the accelerators are observed to be somewhat higher when carbon black is present. This is readily explained by the fact that, on milling, the batch temperature is increased by the presence of the carbon black, the chemical combination of sulfur from the accelerator thereby being enhanced.

The variation of combined sulfur with time of vulcanization is demonstrated by the data of Table V for several samples containing the two Butyl polymers of Table I. These compositions were prepared mainly for the purpose of securing analytical data for a few more or less representative mixtures, and are therefore not to be construed as indicative of the best known compounding formulas. The values of combined sulfur given in Table V are probably somewhat higher than would be found normally, since the samples were unfortunately allowed to remain in the laboratory for about 3 months before being extracted and analyzed. The data of Table V nevertheless prove that 8 hours of extraction with methyl ethyl ketone is ample for the removal of extractable sulfur from the compositions listed. The results furthermore serve to show that, as might be expected, the percentage of combined sulfur increases with time of cure; and for a given time of

cure, it tends to be greater for the polymer containing the greater proportion of chemical unsaturation.

In order to learn how much sulfur remained in the ash of the extracted vulcanizates of Table V, the extracted portions of composition I (prepared with Butyl 1) and of composition II (prepared with Butyl 2) were washed in the sulfur apparatus in the regular manner, and the sulfur contents of the residual ash determined. The results (Table VI) show that the amount of sulfur retained by the ash constituents is about 0.01%, which is practically negligible except in analyses requiring the greatest possible accuracy.

## SUMMARY

Methyl ethyl ketone has been shown to be a much more effective solvent than acetone for the removal of extractable sulfur and certain accelerators from Butyl rubber vulcanizates in a standard Soxhlet extraction. The use of this extraction agent, together with combustion of the original and extracted samples in the Braun-Shell sulfur apparatus, provides a satisfactory procedure for determining the amounts of total and combined sulfur in Butyl compositions.

Table V. Dependence of Combined Sulfur on Time of Cure for Butyl Vulcanizates

(Methyl ethyl ketone extractions)					
Composition		Time of	Time	Sulfur	Remaining
		Cure at 152° C.	of Ex- traction	in Sample	Butyl 1 Butyl 2
		Min.	Hours	%	%
I					
Butyl	100	0	8	Sample lost	0.07
Zinc oxide	2	0	16	0.05	0.03
Sulfur	1.5	30	8	0.27	0.52
Tetramethylthiuram disulfide	1	30	16	0.22	0.50
		60	8	0.38	0.71
		60	16	0.34	0.67
II					
Butyl	100	0	8	0.14	0.17
Zinc oxide	5	0	16	0.14	0.14
Sulfur	1.5	40	8	0.33	0.35
Stearic acid	3	40	16	0.32	0.35
Tetramethylthiuram disulfide	1	60	8	0.49	0.63
Carbon black	50	60	16	0.50	0.61
III					
Butyl	100	0	8	0.14	0.16
Zinc oxide	5	0	16	0.14	0.16
Sulfur	3	40	8	0.32	0.30
Stearic acid	3	40	16	0.35	0.30
Tetramethylthiuram disulfide	1	60	8	0.52	0.63
Carbon black	50	60	16	0.54	0.63

Table VI. Ash Analysis Data for Several Extracted Vulcanizates

Composition	Time of Cure at 152° C. Min.	Time of Extraction Hours	S in Ash %
I (Butyl 1)	60	8	0.012
	60	16	0.014
II (Butyl 2)	60	8	0.012
	60	16	0.013

## ACKNOWLEDGMENTS

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# Determination of Vitamin A and Carotene in Milk

## A Rapid Extraction Procedure

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A rapid procedure for the extraction and determination of vitamin A and carotene in milk is described. Two volumes of milk mixed with 3 volumes of alcoholic potassium hydroxide are allowed to stand for 3 hours at room temperature. The mixture is then extracted twice with ether and the vitamin A and carotene are determined by means of the Carr-Price reaction and with the aid of an Evelyn photoelectric colorimeter.

THE importance of milk as a source of vitamin A makes a rapid and accurate method for the determination of vitamin A and carotene in milk desirable. Earlier procedures which involved butterfat as a starting material (2, 5) were improved by Wilkie (13) and Olson *et al.* (11) by the use of direct extraction procedures based on a modification of the Roesse-Gottlieb method (1) for the determination of fat in milk. Willstaedt and With (14) and Chevallier and Manuel (3) used an ether extraction of milk after incubation for 2 days with aqueous potassium hydroxide.

The method of Olson *et al.* (11) gives satisfactory results, but requires considerable time. Consequently, an attempt was made to develop a more rapid extraction procedure. It was found possible to extract the vitamin A and carotene successfully from whole milk which had been treated with alcoholic potassium hydroxide. The details of this extraction procedure and the subsequent estimation of the vitamin A and carotene with an Evelyn photoelectric colorimeter are described herewith. An abstract of a method of Koehn (10) is somewhat similar to that described here, but does not give details of the procedure.

### REAGENTS AND SPECIAL EQUIPMENT

**POTASSIUM HYDROXIDE SOLUTION.** Add 10 ml. of distilled water to 20 grams of potassium hydroxide (U.S.P. pellets), mix until dissolved, and shake with 90 ml. of absolute ethyl alcohol.

**ANTIMONY TRICHLORIDE SOLUTION.** Rapidly weigh and transfer 20 grams of antimony trichloride, reagent grade, to a brown glass-stoppered bottle and add 100 ml. of chloroform (U.S.P. grade or better). Solution may be hastened by breaking up the lumps with a stirring rod. Filter on a rapid qualitative paper before use.

**ACIDIFIED ALCOHOLIC WASH SOLUTION.** Add 1 ml. of hydrochloric acid, c.p., to 100 ml. of ethyl alcohol and make to 1 liter.

**OTHER REAGENTS.** Petroleum ether (Skellysolve B). Diethyl ether (U.S.P. grade or better); for exact work peroxide-free ether should be used. Chloroform, U.S.P. Absolute ethyl alcohol, aldehyde-free. Anhydrous sodium sulfate, reagent grade. Acetic anhydride, reagent grade.

**RAPID-DELIVERY PIPET (9-ml.).** A convenient, rapid delivery pipet may be constructed by cutting off a 10-ml. pipet, constricting the tip to 2.5-mm. inside diameter, and recalibrating for the rapid delivery of 9.0 ml. The pipet is joined to a three-way stopcock for regulation of filling and delivery and is attached to a rubber suction bulb.

**OTHER EQUIPMENT.** Evelyn colorimeter and tubes, 150- to 200-ml. glass-stoppered pear-shaped separatory funnels, 75-ml. Pyrex test tubes. Y-tube filled with rubber stoppers for evaporation of solvents.

### PROCEDURE

**EXTRACTION.** Thirty milliliters of the alcoholic potassium hydroxide solution are added to a 20.0-ml. sample of fresh, whole milk in a separatory funnel. The contents are mixed by brief, vigorous shaking, and allowed to stand for 3 hours. A yellow coloration which develops does not interfere. Then 25 ml. of diethyl ether are added and the separatory funnel is stoppered tightly and shaken vigorously for 1 minute. After separation

the lower layer is drawn off into a second separatory funnel. Any small amount of emulsified material is retained. The residue in the second funnel is extracted by shaking vigorously for one minute with 18 to 20 ml. of ether. Vapors formed are allowed to escape by carefully opening the stopcock of the inverted funnel. After separation the lower layer is discarded.

To the first separatory funnel 75 ml. of distilled water are added and mixed with the ether extract by inverting the funnel once. The water layer is drawn off into the second separatory funnel, extracted by shaking vigorously for one minute with the ether already present, then discarded. The ether extract in the first funnel is washed by shaking briefly with about 10 ml. of the acidified alcoholic wash solution. The wash mixture is drawn off and shaken with the ether in the second funnel, then discarded. Three milliliters of petroleum ether are added to each funnel to reduce the water content. The extract in the first funnel is washed twice more with 10-ml. portions of the wash mixture and the washings are extracted singly in the second funnel, then combined and allowed to stand for 15 to 20 minutes. Any water settling out is carefully removed.

The extract is then transferred to a 75-ml. Pyrex test tube. The solvents are removed by attaching two such tubes to a vacuum pump (equipped with a water trap) by means of a Y-tube. The vacuum is slowly increased with continual gentle rotatory shaking of the inclined tubes in a water bath at 30° to 40° C. until it is possible to apply full vacuum without excessive frothing of the sample. Then the tubes are heated with continual shaking in a bath at 60° to 70° C. until all solvents are removed but no longer. The tubes are cooled in a beaker of cold water, the vacuum is released, and 5.0 ml. of ether (accurately measured) are added. The residue is dissolved and shaken with 5 ml. of saturated sodium chloride solution, then 10.0 ml. of petroleum ether (accurately measured) are added, and the contents are shaken vigorously. After standing for several minutes the extracts should be crystal clear. If not, the tubes need to be shaken again.

**ANALYSIS.** A 10.0-ml. aliquot is transferred to an Evelyn colorimeter tube, and the total carotenoids are measured using the 440 filter. Then the colorimeter tube is attached to a vacuum pump, and the solvent is evaporated as before. The residue is dissolved in 1.0 ml. of chloroform. A drop of acetic anhydride is added to remove any traces of water. The tube is placed in the colorimeter, a shield or paper towel is placed over the colorimeter to protect it from the reagent used, and 9.0 ml. of 20 volumes % antimony trichloride in chloroform are added from a rapid delivery pipet. The maximum deflection which occurs using the 620 filter is measured. For further details on the use of an Evelyn photoelectric colorimeter for carotene and vitamin A determinations the papers of Kimble (9) and of Dann and Evelyn (4) should be consulted.

**CALCULATIONS.** The calculations and expression of results may be made according to Kimble (9) or Dann and Evelyn (4). However, the authors prefer to express the results in terms of micrograms on the basis of the following calibration of the colorimeter:

The carotene readings are compared to a standard curve prepared by readings with solutions of crystalline carotene. However, for routine work a constant may be used for the calculation of carotene if the galvanometer readings are 35 or above. The constant used in the authors' work is  $L_{440} \times 28 =$  micrograms of carotene per 10 ml. of Skellysolve B.

The blue color produced by the Carr-Price reaction follows Beer's law (4) and a constant is evaluated by standardizing the colorimeter with pure vitamin A, or less preferably with a standardized fish oil. The constant used in the present work is  $L_{620} \times 13.2 =$  micrograms of vitamin A per 10.0 cc. of chloroform. Purified vitamin A and carotene for calibration work may be obtained from Eastman Kodak Co., Rochester, N. Y. For the calculation the vitamin A reading is corrected for the Carr-Price reaction of the carotenoids, using the correction factor of 0.14 (4). The calculation is  $(L_{620} - 0.14 L_{440}) \times 13.2 =$  micrograms of vitamin A per 10.0 ml. of chloroform. For the dilutions used in the above procedure the micrograms of carotene or vitamin A per 10.0 ml. of solvent  $\times 7.5 =$  the micrograms per 100 ml. of whole milk.



Table I. Comparative Analyses of Milk Samples by Different Extraction Procedures

Milk Sample Analyzed	Method of Olson <i>et al.</i>		Present Method	
	Carotene $\gamma/100$ ml.	Vitamin A $\gamma/100$ ml.	Carotene $\gamma/100$ ml.	Vitamin A $\gamma/100$ ml.
1 extraction only				
Holstein, raw	30	32	28	31
	36	24	34	24
	45	31	36	31
Guernsey, raw	102	27	105	25
	101	32	104	27
	103	22	103	18
Brown Swiss, raw	31	36	31	30
Jersey, raw	51	28	50	22
Market, pasteurized	41	35	42	33
	36	38	35	38
	19	16	18	15
Double extraction procedure				
Holstein, raw	25	24	25	23
	30	32	30	32
Guernsey, raw <sup>a</sup>	37	9	35	9
	32	10	30	10
	42	9	41	9
Brown Swiss, raw <sup>a</sup>	11	16	12	16
Jersey, raw <sup>a</sup>	28	21	27	21
Market, pasteurized	22	22	22	22
	41	35	45	36
	36	38	35	38

<sup>a</sup> Milk samples from cows under winter feeding conditions. Other samples are summer milks.

## RESULTS AND DISCUSSION

The principal difficulty encountered in the development of the method was in extracting the vitamin A from the whole milk to which alcoholic potassium hydroxide had been added directly. Several extractions with petroleum ether removed only part of the vitamin A and most of the carotenoids. Extraction with ether under conditions other than those specified above would not remove all the vitamin A. The ratio of alcoholic potassium hydroxide solution and ether used resulted in a complete extraction. With this ratio the first extract was considerably larger than the volume of ether added because part of the alcoholic solution accompanied the ether. The use of two separatory funnels for each sample allows a second extraction of the residue from the first extraction and of the water washings with a minimum of effort and equipment. Extraction of the water washings prevents slight losses of carotene and vitamin A which otherwise occur. The wash mixture of 1 to 1000 hydrochloric acid in 10% alcohol has been found to reduce emulsions to a minimum. It may be used advantageously in other analytical work on vitamin A and carotene where complete removal of all fatty acids is not necessary. Tests with saponified and nonsaponified vitamin A concentrate have shown that this wash mixture results in no destruction or loss of vitamin A when compared to water as a washing agent.

In the development of the method the recovery of vitamin A alcohol added at various steps in the procedure was used as a guide and check. With the method described good recovery (95% or better) of vitamin A alcohol added to the original milk may be obtained, as compared to the same amount of vitamin A added to the final washed extract.

The extraction procedure described was also compared to determinations on the same milk samples with the extraction procedure of Olson, Hegsted, and Peterson (11), except that the samples were saponified in air instead of in nitrogen and the vitamin A and carotene were determined on the washed ether extract of the saponified material with a photoelectric colorimeter, as described above, instead of with a spectrophotometer.

The results of comparative analyses (Table I) show that with a single ether extraction the new method occasionally gave low results for vitamin A. Hence, the double extraction procedure described above was used. The two extraction procedures gave good checks with both pasteurized and raw milks from cows of

various breeds. The variation in the carotene and vitamin A content among breeds and between summer and winter milk is readily evident.

For the majority of the milks analyzed a 20-ml. aliquot will give satisfactory galvanometer deflections. Milk from cows which receive poor feed may be extremely low in carotene and vitamin A and in such cases it is advisable to use a larger sample of milk and concentrate the final extract for the determination.

The carotene determination as given in the procedure is a measure of the total carotenoids in the milk. The carotenoids in cow's milk have been shown to be principally  $\beta$ -carotene (8, 12, 14), whereas human milk may contain considerable amounts of other carotenoids (14). Xanthophylls have been found to comprise about 20% of the total pigments of butter (8). Other yellow pigments which do not have vitamin A activity may be present under certain feeding conditions (8). For more accurate determinations of the  $\beta$ -carotene content the petroleum ether may be freed of interfering pigments by extraction with diacetone alcohol (7) or freed of xanthophylls by extraction with 85% phosphoric acid (6) or by phase separation with 95% methyl alcohol.

## ACKNOWLEDGMENT

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## American Society for Testing Materials Meeting

The 1944 Spring Meeting and Committee Week of A.S.T.M. is to be held in Cincinnati, Ohio, at the Netherland Plaza from February 28 to March 3. The 47th Annual Meeting will be held in New York, N. Y., at the Waldorf-Astoria June 26 to 30 1944.

## Correction

In the article entitled "Benzoin as a Fluorescent Reagent for Zinc [IND. ENG. CHEM., ANAL. ED., **15**, 599 (1943)] on page 600, point 7, should read: Benzoin solution, prepared by dissolving 0.3 gram of benzoin in 100 ml. of hot 95% alcohol.

C. E. WHITE



# Iodometric Determination of Iodates, Bromates, or Permanganates in the Presence of Copper

## Determination of Copper in the Presence of Oxidizing Agents

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KAPUR and Verma (1) have described a method for the iodometric determination of iodate in the presence of copper, based upon the formation of an unreactive copper pyrophosphate complex. The method has the disadvantage that, at the high pH necessary for the formation of the complex, the reaction between iodate and iodide ions is rather slow. Swift and Lee (3) have recently shown that iodate, bromate, or permanganate may be determined in the presence of copper by titration to the iodine chloride end point with standard potassium iodide. The copper present can be determined indirectly by comparing the first result with the total oxidizing power as found by thiosulfate titration of all the iodine liberated from excess iodide in acid solution.

The authors have developed methods by which all the above constituents can be determined, using only a single standard solution (sodium thiosulfate) and the familiar starch-iodine end point. A mixture of the strong oxidant and cupric salt is treated with excess iodide and acid until the reaction is complete. Sodium citrate is then added, forming a stable complex with copper ions, and the copper-iodide reaction reverses quantitatively. The iodine equivalent to the strong oxidant is titrated in the usual manner. After the end point, excess mineral acid may be added, decomposing the copper citrate complex and quantitatively liberating iodine equivalent to the copper. This iodine is titrated, giving a direct determination of both constituents in a single sample and with but one standard solution.

### EXPERIMENTAL

Preliminary experiments showed that the cupric-iodide reaction was quantitatively reversed by excess of neutral oxalates, tartrates, and citrates. A slightly soluble complex cupric salt separates from oxalate solutions. Neither oxalate nor tartrate gave clean-cut reversal of the copper-iodide reaction on the addition of acid. Citrate was found to be the most satisfactory reagent.

Approximately 0.1 *N* stock solutions of potassium iodate, potassium permanganate, potassium bromate, copper sulfate, and sodium thiosulfate were used in the investigation. Titrations were performed by adding nearly the equivalent volume of reactant by pipet and the last milliliter or two from a microburet. This technique permitted estimation of the titration volume to within about 0.002 to 0.003 ml. The procedures which follow were adopted after much experimentation.

### IODATE AND COPPER IN ADMIXTURE

**DETERMINATION OF IODATE.** To 20 to 50 ml. of a solution containing 0.5 to 3 milliequivalents of each constituent add 2 ml. of 6 *N* acetic acid and 6 grams of potassium iodide. Let stand 3 minutes, add 20 ml. of 1.0 *M* sodium citrate, and swirl until the solution becomes clear. This quantity of citrate is sufficient for 3 millimoles of copper. If the solution does not clear completely of cuprous iodide, more must be added. If more than a milliequivalent of mineral acid is present in the original solution, it should be neutralized to incipient precipitation of copper hydroxide, and acetic acid added as in the regular procedure. The citrate should be tested, as some samples were found to use up iodine.

Dilute to 200 ml. and titrate with 0.1 *N* sodium thiosulfate, adding starch at the end point. The color change is a sharp, easily seen transition from a deep murky blue to a clear light blue.

**DETERMINATION OF COPPER.** To the solution from the above titration, add 12 ml. of 6.0 *N* sulfuric or hydrochloric acid, wait 2 minutes, and titrate with thiosulfate. Insufficient acid results in an incomplete reaction. Too large an excess causes previous air-oxidation of iodide (catalyzed by copper). Good results are obtained on adding 1 ml. of 6.0 *N* acid for every 2 ml.

of sodium citrate and then just 1 ml. in excess. It is usually advantageous to add a little more starch at the end point. Although the citric acid present tends to sharpen the end point somewhat, the addition of 3 grams of solid potassium thiocyanate just before the end of the titration is strongly to be recommended.

Typical results taken from 15 determinations are shown in Table I. Swift and Lee have shown that the sum of copper and iodate may be determined very accurately by iodometric titration. This may be used as an alternative method for the indirect determination of copper.

Table I. Titration of Iodine Liberated Equivalent to Iodate (or Bromate) and Copper, in Mixtures<sup>a</sup>

0.1 <i>N</i> KIO <sub>3</sub> Ml.	0.1 <i>N</i> KBrO <sub>3</sub> Ml.	Na <sub>2</sub> S <sub>2</sub> O <sub>4</sub> Required Ml.	Error %	0.1 <i>N</i> CuSO <sub>4</sub> Taken Ml.	Na <sub>2</sub> S <sub>2</sub> O <sub>4</sub> Required for Cu Ml.	Error %
25.00	...	29.32	+0.03	5.00	4.86	0.00
25.00	...	29.32	+0.03	5.00	4.87	+0.2
10.00	...	11.69	-0.26	25.00	24.31	+0.04
10.00	...	11.70	-0.17	25.00	24.31	+0.04
...	25.00	25.28	-0.04	5.00	5.08	-0.2
...	25.00	25.29	0.00	5.00	5.09	0.0
...	25.00	25.27	-0.08	25.00	25.49	+0.08
...	25.00	25.31	+0.08	25.00	25.47	0.00

<sup>a</sup> Different thiosulfate solutions were used in first and last four experiments.

### BROMATE AND COPPER IN ADMIXTURE

The procedure for bromate is the same as for the determination of iodate except that 6 *N* hydrochloric acid is used instead of acetic acid. It is necessary to add 2 ml. for each 30 ml. of the original sample in order to obtain accurate results. Copper is determined as before; however, the hydrochloric acid added for the bromate reaction must be subtracted from the amount to be added to decompose the copper complex. Typical results are shown in Table I.

It was found that in a phthalate buffer of pH 5, copper reacts quantitatively with iodide without interference from bromate present. This permits a very simple determination of copper in the presence of bromate.

To 20 to 50 ml. of the neutral sample solution, add 20 ml. of a 0.1 *M* pH 5 phthalate buffer, and 3 grams of potassium iodide. Titrate with sodium thiosulfate to a starch end point, adding 3 grams of potassium thiocyanate just before the color change. If free acid is present in the original solution, remove it by dropwise addition of ammonia until precipitation of copper hydroxide just begins.

The procedure is accurate—for example, three samples of 20 ml. of approximately 0.1 *N* copper sulfate in the presence of 10 ml. of 0.2 *N* potassium bromate gave titration values of 19.01, 19.00, and 19.00 ml., the value being 19.00 in the absence of bromate. The authors have verified the observation of Swift and Lee that the sum of bromate and copper may accurately be determined by iodometric titration in acid medium.

### PERMANGANATE AND COPPER IN ADMIXTURE

The same procedures may be used as in the iodate and copper determinations, except that the original solution is acidified with sulfuric acid and the presence of this acid must be taken into account in the copper determination. The results are, however, 0.3 to 0.5% low for permanganate and correspondingly high for copper—for example, two mixtures of 20 ml. of 0.1 *N* permanganate and 5 ml. of 0.1 *N* copper gave



titration values of 20.79 and 20.76 ml. (20.84 theoretical) for the permanganate and 4.93 and 4.93 ml. (4.86 theoretical) for the copper, respectively. The sum of the two, determined as above or directly, is in good agreement with the theoretical.

The authors have investigated the source of the error and found that, while neither manganous nor citrate ions affect the titer of an iodine solution, the two together cause a noticeable decrease. A small amount of the manganous citrate complex is evidently oxidized by iodine to the manganic complex, which is unstable in the presence of mineral acids. Attempts at substantially increasing the accuracy of the method by variation of the conditions were unsuccessful. Therefore, the authors recommend that the sum of the two oxidants be determined (3) and the copper estimated in a separate sample. This is easily accomplished if the permanganate is first reduced in acid medium by dropwise addition of saturated ferrous ammonium sulfate solution and any excess of the latter is removed by boiling with a little bromine water. Copper is then determined by the method of Park (2), in which interference from iron is prevented by the

addition of fluoride. The accuracy of this procedure is indicated by the following results: Copper was determined in mixtures of 20 ml. of 0.1 *N* copper sulfate and 20 ml. of 0.1 *N* potassium permanganate; the titration values were 20.17, 20.19, and 20.16 ml. of thiosulfate, the theoretical being 20.19, indicating an average error of less than 0.1%. For a direct, accurate determination of small amounts of permanganate in the presence of copper, the method of Swift and Lee is probably the most convenient.

#### ACKNOWLEDGMENT

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## Cupriethylene Diamine as a Solvent for Precise Determination of Cellulose Viscosity

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**A modification of the tentative standard A.C.S. method for the precise determination of cellulose viscosity has been developed, using a new solvent—cupriethylene diamine.**

**A** TENTATIVE standard method for determining the viscosity of cellulose in cuprammonium hydroxide was presented by the Committee on the Viscosity of Cellulose, Division of Cellulose Chemistry, at the 74th Meeting of the AMERICAN CHEMICAL SOCIETY, September, 1927. This was to a large extent a composite of methods which were in daily use by large manufacturers of cellulose derivatives and was based upon the wealth of experience gained in the research and control laboratories of these organizations.

The present paper has to do with a method involving a new solvent and a different method of manipulation, which is offered as a rapid and precise method of measuring cellulose viscosity.

That cuprammonium hydroxide is by no means the ideal dispersing agent for the determination of cellulose viscosity is evidenced by the many proposals in the literature for the use of other means. A solution of cupriethylene diamine has been suggested. This solvent is easily prepared and adjusted to constant composition, it may be stored under proper conditions at room temperature, and it shows little evidence of spontaneous decomposition over long periods of time. When cellulose is dispersed in cupriethylene diamine solvent, any considerable amount of atmospheric oxygen during the preparation of the dispersion must be avoided, but these dispersions are much less sensitive to the effects of atmospheric oxygen than are dispersions of cellulose in cuprammonium solvent. When cuprammonium is used as a solvent, it is necessary to use extreme caution to prevent the degrading effects of even minute amounts of oxygen while bringing about cellulose dispersion. The tentative A.C.S. method states that hydrogen or nitrogen must be especially purified to free it from traces of oxygen.

In dispersions of cellulose in cupriethylene diamine solvent, ordinary commercial nitrogen containing about 0.5% oxygen may be used with no appreciable effect, provided dispersion is brought about with reasonable rapidity. Furthermore, a cupri-

ethylene diamine solvent 0.5 molar in copper concentration is a much more efficient dispersing agent than any of the standard cuprammonium hydroxide solvents now in use.

Strauss and Levy (2) were the first to describe the successful use of cupriethylene diamine solutions as a solvent in the determination of cellulose viscosity. They pointed out the desirability of using 0.5 molar copper concentrations and gave clear directions for preparing the solvent. As a result of experience extending over nearly two years, several modifications and refinements of their method have been worked out and standardized.

The evolution of the method for rapid viscosity determination has been covered by the author (1). This paper describes more fully the method used for precise viscosity determination where a high degree of precision is required and gives details of the present rapid method used as mill control.

Most of the work done in the author's research laboratory has to do with wood cellulose, the viscosity of which, at 1% concentration, is such that the time of fall of the 1/16-inch aluminum sphere in the standard viscometer is between 5 and 30 seconds. Under these conditions there will be no appreciable change in temperature during the time required for the ball to pass between the two etched marks and it is only necessary to determine the temperature immediately after taking the time of fall of the sphere and apply the temperature correction factor. In dealing with high-viscosity pulps where the time of fall of the standard sphere would exceed 30 seconds, it is desirable to bring the contents of the viscometer to 25° C. by means of constant-temperature water jacket or to run viscosities at lower cellulose concentration. The author hesitates to apply the Farrow and Neale equations for converting viscosities of solutions at lower concentration to the standard 1% concentration because these equations apply only over very narrow limits of concentration.

#### PRECISE METHOD

An unpressed sheet of pulp is air-dried to the point where it is in moisture equilibrium with the air of the balance room



which it is weighed. It is then cut into narrow strips approximately  $0.3 \times 3.0$  cm. and the moisture on the cut-up sample is determined in the usual way, the balance of the sample being placed in an air-tight container while the moisture content is being determined. Two hundred and fifty milligrams of the pulp on an oven-dry basis are weighed and introduced into the solution bottle, 15 ml. of a cupriethylene diamine solution adjusted to 0.167 copper molarity are added, and the pulp sample is thoroughly wetted with this solution. Ten milliliters of the cupriethylene diamine solution, adjusted to 1.000 copper molarity, are then added, the air in the bottle is swept out by a stream of nitrogen, and the bottle is capped and placed in the shaker for 3 minutes. The rest of the determination is carried out by the rapid method which is used as mill control.

In viscosity determinations on cellulose other than wood, the cellulose may be prepared and dried in any convenient form, if it is not too dense to hinder rapid dispersion. Linters and cotton samples may readily be dispersed, but with high-viscosity material several glass beads should be introduced into the solution bottle to aid in the mechanical breaking up of the cellulose.

The viscometer tube described in the previous publication (1) was designed for viscosity determination by the T.A.P.P.I. capillary flow method, but is no longer used for mill control work. At present, the author uses a straight Pyrex tube  $1 \pm 0.005$  cm. in internal diameter, open at both ends and with etched rings 15 or 18 cm. apart, depending upon the conditions of the test. One end of this tube is closed with a rubber stopper. These tubes are much more readily cleaned than those having a capillary at one end.

#### METHOD FOR RAPID VISCOSITY DETERMINATION

The following standard method for rapid viscosity determination is in daily use in the control laboratory. If all the conditions are faithfully followed, a high degree of accuracy in mill control may be expected. However, for precise work, a sample should be prepared and accurately weighed as described above. The precise modification, if properly carried out, should give results which check within 1%.

**PREPARATION OF CUPRIETHYLENE DIAMINE SOLUTION. Materials and equipment.** Chemically pure copper sulfate crystals, 28% ammonia, 20% sodium hydroxide solution, and technical grade ethylene diamine, approximately 70%. Stock solution bottles, heavy enough to withstand vacuum obtained with water pump and a pressure of 2 pounds per square inch. These bottles are equipped with two-hole rubber stoppers carrying inlet and outlet glass tubes equipped with short lengths of rubber tubing and pinchclamps. The rubber stoppers are wired into the stock solution bottles. A cylinder of nitrogen with suitable pressure-reducing valve which will allow delivery of nitrogen at 2 pounds pressure.

Strauss and Levy (2) have determined the composition of cupriethylene diamine solution and caution that there must be no excess of ethylene diamine exceeding 2 moles of ethylene diamine to 1 mole of copper.

Two hundred and fifty grams of copper sulfate are dissolved in approximately 2 liters of hot distilled water. The solution is heated to boiling and sufficient strong ammonia is added with violent agitation to render the solution faintly alkaline to red litmus paper (about 117 ml. of strong ammonia are required). The bluish-green precipitate of basic copper sulfate is allowed to settle and is washed with hot distilled water by decantation until free from sulfate ions. This will require five or six washings. Cold distilled water is then added to the precipitate to bring the volume up to about 1.5 liters and to this slurry are added 850 ml. of cold 20% sodium hydroxide solution while agitating violently. The light bluish-green precipitate changes to the definitely blue color of cupric hydroxide, and is then washed with cold distilled water by decantation until free of both hydroxyl and sulfate ions.

When precipitating the original copper sulfate solution with ammonia, it is necessary to have the solution at the boiling point in order to get a precipitate of maximum density which can be rapidly and thoroughly washed by decantation. The cupric hydroxide prepared from the original precipitate will also be dense and readily washed free of soluble salts. The washed cupric hydroxide is then made into a thick slurry having a

volume of approximately 500 ml. and transferred to a 1-liter bottle equipped with a rubber stopper carrying two glass tubes, one of which is straight and extends to within approximately 5 cm. of the bottom of the bottle; the other is a right-angle bend which extends just through the rubber stopper. The stopper is wired down and all the air over the slurry in the bottle is removed by exhausting with the vacuum pump and filling with nitrogen at 2 pounds' pressure three separate times. After removal of all air a vacuum is drawn on the bottle and 160 ml. of 70% ethylene diamine are introduced, care being taken to allow no air to enter. The reaction between the cupric hydroxide and the ethylene diamine evolves considerable heat at this point. The contents of the bottle are thoroughly shaken several times over the course of an hour and the solution is then allowed to stand for 12 to 16 hours.

From the stock solution prepared as above, two solutions are prepared for dissolving the pulp samples. One is adjusted to  $1.000 \pm 0.005$  molarity with distilled water and stored under an atmosphere of nitrogen. The second solution is made up from the stock solution and distilled water, adjusted to 0.167 molarity, and stored under an atmosphere of nitrogen.

**TESTING CUPRIETHYLENE DIAMINE SOLUTION. Materials required.** A 250-ml. volumetric flask, 25-ml. pipet, 1.000 *N* hydrochloric acid, and 0.100 *N* thiosulfate.

**Procedure.** After allowing the cupric hydroxide sludge to settle, a 25-ml. sample of the supernatant liquid is made up to 250 ml. A 25-ml. aliquot is acidified with 50 ml. of 4 *N* sulfuric acid, approximately 3 grams of potassium iodide are added, and the solution is titrated with 0.1 *N* thiosulfate to a starch end point. The milliliters of thiosulfate required multiplied by 0.04 equals the molarity of the solution in copper.

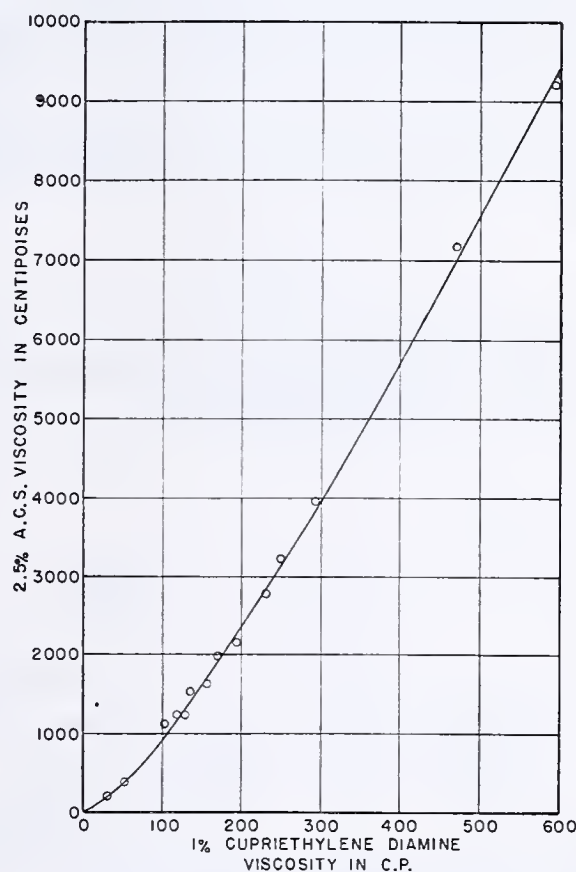


Figure 1

A second 25-ml. aliquot is diluted to approximately 100 ml. with distilled water and titrated with 1 *N* hydrochloric acid to a faint pink coloration, using 2 drops of methyl orange indicator. The solution changes during this titration from dark blue to light blue and finally develops a pink tinge. The pH of this end point, checked from a large number of samples, is between pH 3.2 and 3.3 as determined with a glass electrode. The milliliters of 1 *N* hydrochloric acid required multiplied by 0.4 equals the alkalinity of the solution in hydrogen-ion equivalents.

The reactions taking place in neutralization of cupriethylene diamine with hydrochloric acid are:







En = ethylene diamine,  $\text{C}_2\text{H}_4(\text{NH}_2)_2$

These reactions indicate a theoretical combination of two ethylene diamine molecules with one of copper.

When an excess of cupric hydroxide is in complete equilibrium with dilute ethylene diamine the actual molar ratio is 1.9 moles of ethylene diamine to one mole of copper, according to the method of testing outlined above.

Viscosity tests of various pulps, using cupriethylene diamine liquors having higher than 2 to 1 ratios of ethylene diamine to copper, have indicated the ratio must be kept below 2.00 to 1 by this test to obtain reproducible viscosities. Viscosities have been shown to be too high by approximately 3 to 5% at a 2.1 to 1 ratio and 25 to 30% at a 3 to 1 ratio.

**CALIBRATION OF VISCOSITY TUBES.** *Equipment required.* Viscosity tube,  $1.00 \pm 0.005$  cm. inside diameter, 30 cm. long with etched rings 18 cm. apart;  $1/16$ -inch aluminum balls, plastic-tipped forceps, accurate thermometer reading to  $0.10^\circ\text{C}$ . Constant-temperature bath  $25.00^\circ\text{C}$ ., tube holder in bath. Fluorescent light. Standard viscosity oil. Stop watch reading to 0.1 second.

The thermometer, preferably, should have a certified test point at  $25.0^\circ\text{C}$ . and the temperature of the bath during calibration should be held as close to this point as possible.

*Procedure.* The tubes are filled with standard viscosity oil obtained from the National Bureau of Standards. This oil should be between 200 and 300 centipoises at  $25^\circ\text{C}$ .; the exact value will be indicated by the Bureau of Standards. The tubes are closed and placed in the constant-temperature bath, held at  $25.00^\circ\text{C}$ . for at least an hour before making the determinations. When the tubes have reached constant temperature, a tube is unstoppered and placed in the tube holder in the bath.

The tube holder must hold the tubes in a vertical position, so the falling ball will drop down the center of the tube and not approach the tube wall; otherwise incorrect times of fall will be observed.

Using plastic-tipped forceps, a  $1/16$ -inch aluminum ball is carefully dropped in the center of the tube and the time of fall between the etched lines on the tube is determined to the nearest 0.1 second.

The tube factor is calculated as follows:

$$\eta = Kt(D-d)$$

where

- $\eta$  = viscosity of standard oil at  $25^\circ\text{C}$ .
- $K$  = tube and ball constant
- $t$  = time of fall through 18 cm. of standard oil at  $25^\circ\text{C}$ .
- $D$  = density of aluminum balls = 2.805
- $d$  = density of standard oil at  $25^\circ\text{C}$ .

Then:

$$K = \frac{\eta}{t(D-d)}$$

$$\eta' = t'K(D-d') = t' \frac{\eta(D-d)}{t(D-d)} = t' \frac{1.753\eta}{t(2.805-d)}$$

where

- $d'$  = density of 1% cupriethylene diamine cellulose solution at  $25^\circ\text{C}$ . = 1.052
- $\eta'$  = viscosity of unknown cupriethylene diamine cellulose solution
- $t'$  = time of fall of aluminum ball in unknown solution

**PREPARATION OF PULP SAMPLES.** *Equipment required.* Centrifuge, 7-cm. Büchner funnel, filter paper, 2 suction flasks, moisture teller, weighing bottles, desiccator, 60-ml. flat medicine bottles with plastic screw caps and rubber gaskets.

*Procedure.* A sample of the pulp, from a centrifuge pad or a dried sheet, is weighed so as to get from 0.25 to 0.35 gram of oven-dry pulp. The sample is dispersed in approximately 1 liter of water, filtered on a 7-cm. Büchner funnel with filter paper, and washed with two 25-ml. portions of acetone, the acetone being saved for recovery by distillation.

The acetone-washed pulp is stripped from the filter paper and placed in a "moisture teller" with the heat switch on for exactly 2 minutes, then quickly transferred to a tared weighing bottle, and placed in a desiccator until it can be weighed. After the pulp weight is determined, the pulp is transferred to the 60-ml. flat medicine bottle.

The oven-dry content of pulp after 2 minutes' drying in the moisture teller enables one to calculate the amount of the two reagents necessary for dissolving the pulp to give a 1.0% solution.

Tests have shown that when the above equipment and method are used, the pulp will be 99.5% oven-dry.

With different equipment and different pulp, the moisture content of the pulp, after it comes from the moisture teller, may vary from the 99.5% oven-dry which the author has determined. This figure must be determined for each individual mill under actual working conditions.

**SOLUTION OF CELLULOSE SAMPLE.** *Equipment required.* Shaker, 200 cycles per minute with a 7.5- to 10-cm. amplitude.  $1.00 \pm 0.005\text{ M}$  cupriethylene diamine, solution B.  $0.167\text{ M}$  cupriethylene diamine, solution A. Two 25-ml. pinchcock burets with side tube. Nitrogen cylinder with reducing valve to give 2 pounds pressure.

A table of quantities of solution for weights of samples may be made up according to the oven-dry percentage given by any individual moisture teller by the following formula:

$$0.6\text{ WB} = \text{ml. of } 0.167 \text{ cupriethylene diamine}$$

$$0.4\text{ WB} = \text{ml. of } 1.00 \text{ cupriethylene diamine}$$

where  $W$  = weight of sample and  $B$  = % oven-dry.

*Procedure.* Solution A is added to the bottle from a buret, and the pulp sample is thoroughly wetted. Then solution B is added from a second buret, the bottle is swept out with a stream of nitrogen for at least 15 seconds, and the cap is quickly screwed in place. (The nitrogen is applied to the bottle by means of a 5-mm. glass tube clamped in a vertical position with a rubber tubing connection from the upper end to a nitrogen cylinder with reducing valve which gives approximately 2 pounds per square inch pressure. The 60-ml. bottle is swept free of air by raising it to the glass tube in such a manner that the lower end of the tube extends just below the neck, and the bottle is slowly tipped so as to sweep the nitrogen stream over the surface of the liquid at all corners of the bottle.)

The sample bottle is then shaken by hand or machine for exactly 3 minutes to accomplish solution of the cellulose.

**MEASUREMENT OF VISCOSITY IN CENTIPOISES.** *Equipment required.* Calibrated viscosity tubes,  $1/16$ -inch aluminum balls, viscosity tube viewer with fluorescent light background. Thermometer, reading to  $0.1^\circ\text{C}$ . Plastic-tipped forceps for handling aluminum balls.

*Procedure.* The solution is allowed to stand for exactly 5 minutes to allow escape of any large bubbles entrapped during shaking. Tube is placed in a vertical position so that the standard balls will not approach the sides of the tube as they fall. The time required for a  $1/16$ -inch aluminum ball to fall between the 18-cm. marks on the tube is measured with a stop watch.

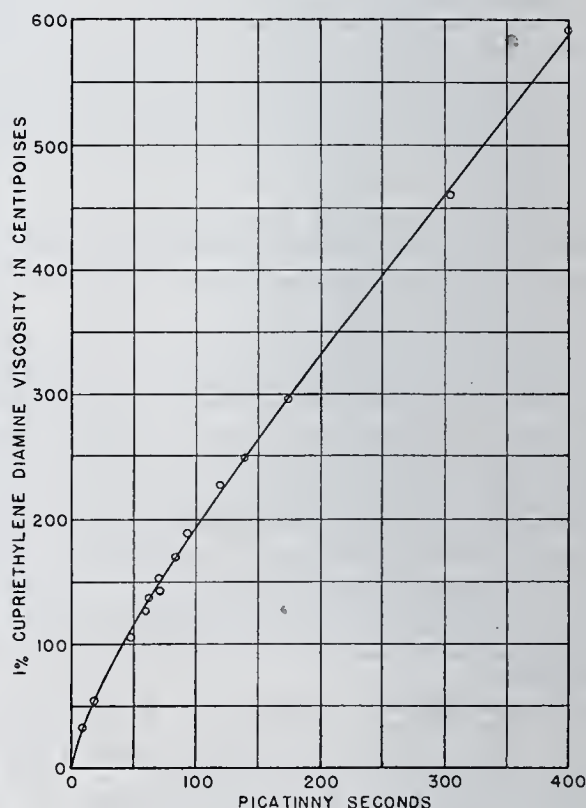


Figure 2



The balls may readily be observed by placing a shielded fluorescent light behind the vertical tube holder. More than one ball should be timed to assure an accurate test.

The temperature of the viscosity solution is measured to the nearest 0.1° C. immediately after determining the falling ball time. It is very important that the temperature of the solution does not change during the time of dropping the balls and measuring the temperature. The viscosity of the solution is determined by the following equation:

$$\log \text{ tube constant} + \log \text{ seconds fall} + \frac{1}{(T - 25^\circ \text{ C.})}(0.01866) = \log \text{ viscosity at } 25^\circ \text{ C.}$$

The temperature correction is added above 25° C. and subtracted below 25° C.

### CONCLUSIONS

The author and his assistants developed this method for use in controlling the preparation of pulp to meet viscosity specifications of the Picatinny Arsenal. These specifications follow the tentative standard A.C.S. method, except that viscosity is expressed in seconds of fall of the standard glass sphere through 10 instead of 15 cm., and for pulp to be used in the manufacture of smokeless powder, the time of fall of the standard glass sphere through 20 cm., instead of centipoises, is used to express viscosity.

Figure 1 shows the relationship between parallel determinations made by the tentative standard A.C.S. method using a 2.5% cellulose concentration and the cupriethylene diamine modification using a 1% cellulose concentration.

Figure 2 shows the relationship between parallel tests made according to Picatinny Arsenal specifications and the cupriethylene diamine method using a 1% cellulose concentration.

For very high viscosity it is advisable to use concentrations under 1%, and for very low viscosity to use concentrations above 1%. In order to express the viscosities in terms of 1% concentration, when concentrations above or below 1% are used, it is necessary to determine the mathematical relationship between concentration and viscosity. So far no universal mathematical relationship has been found which may be used to convert viscosities at concentrations above and below 1% to the viscosity at 1% concentration.

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# Furfural Solution Temperatures of Hydrocarbons

## Evaluation of Mixed Aniline Point Determination and Application of Furfural

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The application of furfural for the determination of miscibility solution temperatures of petroleum fractions and the influence of the composition of the 60° diluent on the results of the mixed aniline point test have been studied. Furfural offers considerable promise; it is nontoxic and is directly applicable to petroleum fractions of high aromatic content, making possible elimination of mixed aniline point determination.

THE solvency characteristics of various petroleum fractions of the type of solvent oil, solvent naphtha, Diesel fuels, various gas oils, etc., constitute an important index as to the properties such products will display in use. The determination of these characteristics has been an important task of the petroleum technologist, and various methods have been devised for their evaluation. Of these methods, those based upon the miscibility solution temperature of the oil and a solvent liquid are among the most important.

The "miscibility solution temperature" is defined as the minimum equilibrium solution temperature for equal volumes of petroleum product and solvent liquid. Since the different classes of hydrocarbons display different solubilities in various solvents, it is possible to obtain an indication of the nature of an oil from its miscibility solution temperature. Of the various types of solvents proposed for this determination aniline (2) has been the most widely used (6). Nitrobenzene (7), benzyl alcohol (3), ethyl alcohol (4), mixtures of acetone and amyl acetate (14), and nitromethane (8) have also been suggested as solvents for this test.

Aniline appears to have been accepted as the standard solvent in determining miscibility solution temperatures of petroleum fractions; however, it has a number of defects which make it desirable to find a more widely acceptable substitute. Aniline is a blood poison and its fumes are readily absorbed. While indi-

vidual response differs, its high toxicity is generally admitted. Further, aniline cannot be used for the determination of the miscibility temperature of high aromatic content petroleum fractions because of its relatively high freezing point.

In an effort to circumvent this shortcoming, the so-called "mixed aniline point" test has been introduced in relatively recent years in this country as a mode for evaluating these materials. The test represents the minimum, equilibrium solution temperature of a mixture of anhydrous aniline and equal volumes of the material under test and "any naphtha having a straight aniline point of 60° C." (11). It has become increasingly apparent that the results of this test are greatly influenced by the chemical composition of the 60° diluent—i.e., by the aromatic-naphthene-paraffin ratio. As a matter of fact the test, as it is now carried out, is completely meaningless; and it will remain so until the composition of the 60° diluent is standardized or the aniline is replaced by a reagent of lower freezing point. This latter alternative would eliminate the use of a diluent and, hence, the need for standardizing it.

The present paper presents a study of the application of furfural for the determination of miscibility solution temperatures of petroleum fractions. A critical study has been made of the influence of the composition of the 60° diluent on the results of the mixed aniline point test.

Manley, McCarty, and Gross (9) in 1933 studied the use of furfural for the solvent extraction of motor oils. They found it to have a high degree of selective solvent action on a wide variety of petroleum fractions, to be stable in closed systems, possessed of a high application temperature (149° C.), and nonpoisonous. Syono (12) determined the furfural miscibility temperatures of a gasoline of 53.8% aromatic hydrocarbon content at various ratios of furfural to test oil. Trimble (13) recently has studied the solvent properties of furfural, chiefly with inorganic materials, although he tested a number of organic compounds including a solvent naphtha. He suggested that furfural could be used to



Table I. Variation of Furfural Point with Aromatic Content

Xylene Vol. %	Xylene Ml.	Petroleum Solvent 1 Ml.	Furfural Ml.	Furfural Point ° C.
0	0	7.0	7.0	110.8
10	0.7	6.3	7.0	102.3
20	1.4	5.6	7.0	93.0
30	2.1	4.9	7.0	83.6
40	2.8	4.1	7.0	73.4
50	3.5	3.5	7.0	62.0
60	4.2	2.8	7.0	48.9
70	4.9	2.1	7.0	32.5
80	5.6	1.4	7.0	13.3
90	6.3	0.7	7.0	-14.8

Table II. Effect on Furfural Point of Varying Volume Ratio of Furfural to Petroleum Solvent 1

Volume Ratio Furfural/ Petroleum Solvent 1	Furfural Ml.	Petroleum Solvent 1 Ml.	Furfural Point ° C.
1:4	2.8	11.2	99.8
1:3	3.5	10.5	105.4
1:2	4.5	9.0	109.4
1:1	7.0	7.0	110.8
2:1	9.0	4.5	108.5
3:1	10.5	3.5	101.6
4:1	11.2	2.8	95.2

distinguish between aliphatic and aromatic hydrocarbons. Dunlop and Peters (5) studied the thermal stability of furfural and in confirmation of Manley *et al.* (9) found that this material possessed high temperature stability. An extensive bibliographic survey of furfural and its derivatives has been published (10).

#### MATERIALS

**FURFURAL.** This reagent was secured from the Eastman Kodak Company and comprised the best grade. It was purified before use by the method of Adams and Vorhees (1).

**PETROLEUM FRACTIONS.** A set of petroleum fractions upon which "aniline points" are normally determined was obtained through the courtesy of the Standard Inspection Laboratory, Standard Oil Development Company, Bayonne, N. J.

Before use, all the hydrocarbons were dried by contact with anhydrous calcium chloride followed by filtration.

Table III. Effect on Furfural Point of Varying Volume Ratio of Furfural to Petroleum Solvent 1 Plus 40% Xylene

Volume Ratio Furfural/Test Oil	Oil Composition			Furfural Point ° C.
	Furfural Ml.	Xylene Ml.	Petroleum solvent 1 Ml.	
1:4	3.0	4.8	7.2	45.0
1:3	3.75	4.5	6.75	52.5
1:2	5.0	4.0	6.0	60.6
1:1	7.0	2.8	4.2	72.8
2:1	9.4	1.9	2.8	76.4
3:1	11.25	1.5	2.25	72.8
4:1	12.0	1.2	1.8	67.5

#### APPARATUS AND PROCEDURE

The apparatus and procedure are essentially those described by Williams and Dean (16) for the determination of aniline points. All test mixtures were prepared volumetrically by means of a buret.

Furfural miscibility temperatures below about 30° C. are obtained with the same apparatus and procedure, except that cooling instead of heating is employed. The cooling medium comprises isopropyl alcohol and dry ice, the dry ice being added slowly to a point slightly below immiscibility. The bath is then allowed to warm spontaneously to the point of miscibility and the temperature recorded.

All recorded furfural miscibility temperatures are the check results of three or more independent determinations which agreed to 0.2° C.

#### METHOD

**STANDARDIZATION OF FURFURAL.** Within the first hour after the distillation of the furfural by the method of Adams and Vorhees (1), its temperature of miscibility with petroleum solvent 1 was determined. This value was designated as the "original

furfural point" and was so marked on the furfural supply bottle. Whenever tests were made on successive or separate days the "furfural point" of the petroleum solvent 1, retained as standard was checked against the supply of furfural on each day that determinations were to be made, and corrections were made on all determinations for the day on the basis of the check test. When the furfural point of the reference petroleum solvent varied by more than 0.5° C. from its original furfural point the supply of furfural was considered unsatisfactory and was not used again until it had been redistilled and restandardized. Present experience indicates that the furfural can be used over several weeks within the limits imposed.

#### EXPERIMENTAL RESULTS

**AROMATIC CONTENT.** The variation of furfural point with aromatic content was determined on a series of synthetic mixtures comprising varying volumes of petroleum solvent 1 and xylene. The data obtained are summarized in Table I and Figure 2.

Table IV. Furfural Points of Various Petroleum Fractions

Sample	Aniline Point <sup>a</sup> ° C.	Furfural Point ° C.
Solvent Oil	73.5	110.8
Solvent Naphtha	59.5	81.0
Transformer Oil	93.0	122.8
Heavy Marine Diesel	53.0	88.4
Lubricating Oil	83.5	120.0
Gas Oil	68.0	99.4
Hydraulic Oil	89.0	122.0

<sup>a</sup> Aniline points were determined by Standard Inspection Laboratory, Standard Oil Development Co., Bayonne, N. J.

**RELATIVE VOLUME RATIO OF FURFURAL TO OIL.** The effect on the furfural miscibility temperatures of different relative volumes of furfural to test oil was determined from the standard petroleum solvent 1 and petroleum solvent 1 containing 40% by volume of xylene. The data obtained are summarized in Tables II and III and Figure 1.

**FURFURAL POINTS OF VARIOUS PETROLEUM FRACTIONS.** Table IV summarizes the results obtained upon a series of petroleum fractions. These fractions represent typical products upon which aniline points are ordinarily determined.

**SUBSTITUTION OF FURFURAL FOR MIXED ANILINE POINT DETERMINATION.** Table V summarizes the list of petroleum and aromatic materials used in the evaluation of the mixed aniline point determination. Distillation boiling ranges, approximate aromatic contents, and aniline and furfural points are presented for these materials where possible.

Table VI summarizes the data obtained in determining the variation in mixed aniline point with composition of the "60 diluents" which were prepared by various combinations of the petroleum solvents whose properties are presented in Table VII. Table VII presents a comparison of the mixed aniline and furfural points of the aromatic petroleum naphthas studied in Table VI.

Table V. List of Petroleum and Aromatic Materials Used in Mixed Aniline Point Evaluation

Material	Distillation Range ° C.	Aromatics Vol. %	Aniline Point ° C.	Furfural Point ° C.
Petroleum Solvent 1	203.0-265.0	0	81.8	111.5
Petroleum Solvent 2	155.0-210.0	...	58.3	87.2
Petroleum Solvent 3	97.5-139.0	69.0	-30.4	-4.1
Petroleum Solvent 4	136.0-155.0	91.5	...	-61.5
Petroleum Solvent 5	220.0-355.0	0	80.3	113.3
Petroleum Solvent 6	175.0-300.0	5	69.3	98.0
Petroleum Solvent 7	175.0-275.0	12	63.5	94.5
Petroleum Solvent 8	138.5-181.5	84.5	...	-42.0
Petroleum Solvent 9	186.0-216.0	88.0	...	-18.2
Petroleum Solvent 10	98.0-134.0	45.0	13.4	40.8
Benzene, C.P.	80.1	100	...	...
Toluene, C.P.	109.0-111.0	100	...	-60.7

<sup>a</sup> Crystallization temperature.



Table VI. Variation in Mixed Aniline Point with Composition of 60° Diluent

Diluent No.	Composition of 60° Diluent Blend				50/50 Blend of Diluent with:												
					Petroleum solvent 10		Petroleum solvent 3		Petroleum solvent 9		Petroleum solvent 8		Petroleum solvent 4		Benzene		Toluene
	Aro-matic	Aniline point	Aro-matic	Mixed aniline point	Aro-matic	Mixed aniline point	Aro-matic	Mixed aniline point	Aro-matic	Mixed aniline point	Aro-matic	Mixed aniline point	Aro-matic	Mixed aniline point	Aro-matic	Mixed aniline point	
	Vol. %	° C.	Vol. %	° C.	Vol. %	° C.	Vol. %	° C.	Vol. %	° C.	Vol. %	° C.	Vol. %	° C.	Vol. %	° C.	
I	6.7% Petroleum solvent 1	..	60.1	..	40.1	..	25.4	..	21.8	..	17.6	..	14.7	..	10.1	..	9.1
II	93.3% Petroleum solvent 2																
	72.0% Petroleum solvent 1	19	60.3	32.0	43.4	44.0	28.8	53.5	25.7	51.7	23.8	55.2	20.1	59.5	15.1	59.5	15.9
III	28.0% Petroleum solvent 3																
	76.0% Petroleum solvent 1	22	60.2	33.5	40.7	45.5	28.0	55.0	24.6	53.2	21.4	56.7	19.0	61.0	15.8	61.0	14.9
IV	24.0% Petroleum solvent 4																
	6.7% Petroleum solvent 5	..	60.3	..	39.8	..	25.7	..	21.9	..	17.8	..	14.2	..	10.0	..	8.5
V	93.3% Petroleum solvent 2																
	73.3% Petroleum solvent 5	18	60.0	31.5	42.2	43.5	27.2	53.0	25.0	51.2	21.2	54.7	18.9	59.0	16.1	59.0	14.9
VI	26.7% Petroleum solvent 3																
	77.3% Petroleum solvent 5	21	59.9	33.0	40.0	45.0	28.0	54.5	25.2	52.7	22.4	56.2	18.2	60.5	17.4	60.5	14.3
VII	22.7% Petroleum solvent 4																
	13.3% Petroleum solvent 6	..	60.0	..	39.6	..	25.2	..	22.0	..	17.9	..	14.4	..	10.3	..	8.6
VIII	86.7% Petroleum solvent 2																
	26.7% Petroleum solvent 7	..	60.2	..	39.9	..	25.1	..	22.5	..	18.4	..	15.2	..	11.2	..	9.6
IX	73.3% Petroleum solvent 2																
	94.6% Petroleum solvent 7	15	60.2	30.0	40.9	42.1	27.4	51.5	22.9	49.8	19.2	53.3	16.2	57.5	12.9	57.5	11.1
X	5.4% Petroleum solvent 3																
	96.0% Petroleum solvent 7	15	60.4	30.0	39.6	42.1	26.2	51.5	23.2	49.8	19.6	53.3	16.6	57.5	13.6	57.5	11.4
	4.0% Petroleum solvent 4																
Maximum variation in mixed aniline point, ° C.					3.8		3.7		3.9		6.2		5.9		7.4		7.4

## DISCUSSION

The application of furfural for the determination of miscibility solution temperatures of petroleum fractions appears to offer considerable promise over the solvents now generally employed. Furfural is nontoxic, stable under proper conditions of storage, and applicable to a wide range of petroleum fractions. The point of complete miscibility is easily and readily determinable even in darkly colored petroleum fractions.

Aniline is completely miscible in petroleum fractions containing approximately 50% or more of aromatic hydrocarbons.

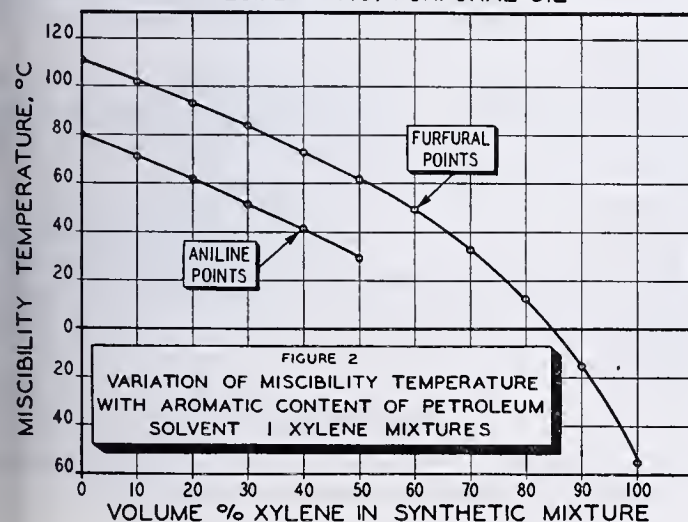
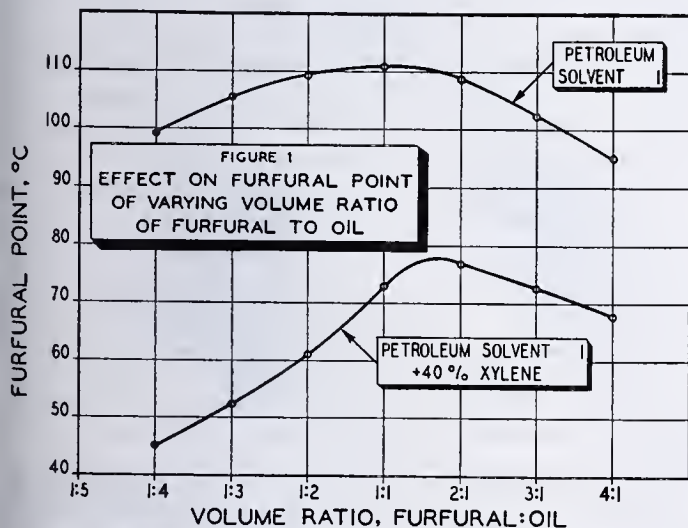


Table VII. Comparison of Mixed Aniline and Furfural Points of Aromatic Petroleum Naphthas

Material	Mixed Aniline Point		Furfural Point ° C.
	Maximum ° C.	Minimum ° C.	
Petroleum Solvent 3	28.8	25.1	-4.1
Petroleum Solvent 4	20.1	14.2	-61.3
Petroleum Solvent 8	23.8	17.6	-42.0
Petroleum Solvent 9	25.7	21.8	-18.2
Petroleum Solvent 10	43.4	40.0	40.8
Benzene	17.4	10.0	..
Toluene	15.9	8.5	-60.7*

\* Crystallization point.

Table VIII. Effect of Aromaticity

Xylene in Petroleum Solvent 1 %	Furfural Point ° C.	Aniline Point ° C.	Difference ° C.
0	110.8	79.6	31.2
10	102.3	70.6	31.7
20	93.0	61.2	31.8
30	83.6	50.6	33.0
40	73.4	40.5	32.9
50	62.0	28.9	33.1

For these materials it is necessary to employ the so-called "mixed aniline point" determination. This determination becomes a relatively complex proceeding in comparison with the furfural miscibility determination, which can be carried out directly.

The critical study which has been made of the influence of the composition of the 60° diluent on the results of the mixed aniline point has shown the extent of the variation which can occur when using an unstandardized diluent. It is obvious that an unlimited number of 60° diluents can be prepared; all that is necessary is two hydrocarbon fractions, one having an aniline point above 60° and the other below. On the other hand, a direct miscibility solution temperature can be determined on these materials by the use of a lower freezing point solvent, such as furfural.

The furfural miscibility temperature, as indicated by Figure 1, is dependent upon the relative volumes of furfural to test oil. In this respect its behavior is similar to that of aniline and other solvents (15). For practical determinations of miscibility the standard procedure of employing equal volumes of furfural to test oil is suitable.

Variation in aromaticity for a given paraffinic and aromatic fraction will yield differences between furfural and aniline miscibility temperatures of fairly constant value as indicated in Table VIII.



The average difference is 32.3. While it appears that for certain petroleum fractions this difference may vary over a much wider range; nevertheless, it is interesting to note that for the varied petroleum fractions studied in Table IV, the average difference between the furfural and aniline miscibility temperatures is 32.1. The large variations from the mean which are encountered are involved with the composition of the paraffinic fraction although work with pure hydrocarbons will have to be carried out in order to establish this point. Work is in progress in that direction and will be reported in a future paper.

### CONCLUSIONS

The aniline point method, though widely used, has the disadvantage of using a toxic reagent and limited applicability to low aromatic content petroleum fractions. A search of the literature and an experimental study have indicated that furfural offers considerable promise as a reagent for the determination of miscibility solution temperatures. It is nontoxic and is directly applicable to petroleum fractions of high aromatic content, making possible the elimination of the mixed aniline point determination.

### ACKNOWLEDGMENT

Opportunity is taken to thank the Standard Inspection Laboratory of the Standard Oil Development Company for comment

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# A Color Test for the Carbonyl Group

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THE carbonyl group is usually identified by reaction with a hydrazine derivative, the appearance of the hydrazone precipitate constituting a positive test (2). This reaction is both sensitive and specific, but it lacks the simplicity and rapidity which are characteristic of a color test.

Hydroxylamine hydrochloride reacts with carbonyl groups, forming the oxime and liberating hydrochloric acid. If a suitable acid-base indicator is added to the reaction mixture, the liberation of hydrochloric acid is accompanied by a color change; thus, the reaction serves as a color test for the carbonyl group. When combined with a specific test for the aldehyde group (1) the presence of ketones can be established in the absence of aldehydes.

### REAGENTS

**REAGENT A.** Prepare a solution containing 5 grams of hydroxylamine hydrochloride per liter of 95% alcohol. To 1 liter of this solution add 3 ml. of Bogen (Coleman and Bell, Norwood, Ohio) or Grammercy (Fischer Scientific Co., 711 Forbes St., Pittsburgh, Pa.) universal indicator. If necessary, add dropwise sufficient alcoholic sodium hydroxide to change the color to a bright orange (pH 3.7 to 3.9), taking care not to add too much base. The reagent, now ready for use, is stable for long periods of time.

**REAGENT B.** Prepare a solution of the indicator by adding 3 ml. of the latter to 1 liter of 95% alcohol.

### PROCEDURE

Place 0.5 to 1 ml. of Reagent A in a small test tube, and add a drop, or a few crystals, of the compound to be tested. A change in color from orange to red is a positive test. If no pronounced color change occurs, heat the contents of the test tube to boiling and allow a few minutes for the reaction to take place.

If the compound is suspected of being slightly acidic or basic, add 4 or 5 drops to 0.5 ml. of Reagent B and bring to the color of Reagent A by adding dropwise dilute sodium hydroxide or hydrochloric acid. Then carry out the test by adding this solution to Reagent A.

### EXPERIMENTAL

The test was applied to the following aldehydes and ketones:

Aldehydes	Ketones
Formaldehyde	Acetone
Acetaldehyde	Methyl ethyl ketone
Propionaldehyde	Acetophenone
Butyraldehyde	Cyclohexanone
Chloral	Biacetyl
Benzaldehyde	Pyruvic acid
Salicylaldehyde	Ethyl acetoacetate
Vanillin	Diacetone alcohol
Citral	Pinacolone
Citronellal	Mesityl oxide
Furfural	Benzophenone
Acetal	Benzil
Cinnamaldehyde	Benzoin
	Camphor

An immediate change in color was shown by all except the last four ketones; in these cases, heating was required. Sugar quinones, and compounds such as the benzoyl benzoic acids fail to give a test.

**INTERFERENCES.** No noncarbonyl compound was found which gave a positive test. Because of buffer action, excessive amounts of amines or acids obscure the test and must be separated from the carbonyl compounds.

**SENSITIVITY.** A positive test is obtained with aldehydes whose concentration in the test solution is 0.01 M to 0.02 M, the aldehydes of lower molecular weight being most sensitive. The sensitivity to ketones varies greatly with the type and molecular weight, varying from 0.02 M for acetone to about 0.25 M for benzophenone.

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# Colorimetric Determination of Iron with Disodium-1,2-dihydroxybenzene-3,5-disulfonate

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A new sensitive, stable, and widely applicable reagent for the colorimetric determination of ferric iron is presented. The nature of the reaction and chemical behavior of the ferric complex has been studied both visually and spectrophotometrically. The reagent may be used in either acid or alkaline medium. In alkaline solution (pH 9 to 10), it is sensitive to one part of iron in 200,000,000 parts of solution when observations are made in Nessler cylinders (50-ml., tall-form); in acid solution (pH 3.5 to 4.5), the sensitivity

is one part in 30,000,000. The colored complexes (red in alkaline medium, blue in acid) obey Beer's law over the useful range of iron concentrations.

A variety of materials has been analyzed with a high degree of accuracy. The number of interfering ions is small. Analyses may be carried out in the presence of fluorides, phosphates, tartrates, citrates, oxalates, etc. Procedures are given for the use of the reagent.

MANY *o*-dihydroxybenzene derivatives react with ferric iron, resulting in the formation of intensely colored compounds which may be used in the colorimetric determination of iron (2-5, 7, 9, 10). This paper introduces disodium-1,2-dihydroxybenzene-3,5-disulfonate, from this class of compounds, as a new colorimetric reagent for ferric iron. The new reagent is exceedingly sensitive and subject to remarkably little interference by other ions. Ferric iron may be determined colorimetrically with this reagent in the presence of fluorides, phosphates, tartrates, citrates, oxalates, and other ions that normally interfere seriously with colorimetric iron determinations. The procedure for its application in analysis is simple and the results obtained by its use are highly satisfactory.

It was observed during a systematic investigation of a series of medicinals, for their color or precipitate formation with about eighty inorganic ions, that "fuadin" (fouadin), an antimony complex of sodium catechol disulfonate, gave an intense blue color in acid solutions of ferric iron. The blue color was found to change to an intense red when the solution was made alkaline. Further investigation revealed that the active constituent of fuadin is disodium-1,2-dihydroxybenzene-3,5-disulfonate, one of the compounds from which fuadin is formed. An extensive study of the reaction of this compound with ferric iron showed that it is well suited for the colorimetric determination of iron. The iron complex is highly soluble in water and is stable to light; the color intensity is deep, permitting detection of a very small amount of iron; and the color reaction is practically specific. Moreover, the reagent is colorless in aqueous solution, a very desirable property of colorimetric reagents. A search of the literature did not reveal that either fuadin or disodium-1,2-dihydroxybenzene-3,5-disulfonate had been reported as a colorimetric reagent for iron.

## APPARATUS AND SOLUTIONS

**INSTRUMENTS.** Spectrophotometric measurements were made with a Beckman quartz spectrophotometer, Model D, using 1-cm. Correx glass transmission cells, at a spectral band width of 5 m $\mu$ .

All pH measurements were made with a Beckman glass electrode pH meter, Model G.

Visual observations were made in matched 50-ml. Nessler cylinders (tall-form) and the Yoe roulette comparator using 100-ml. tubes (160 mm., 12).

**ACIDS.** Unless otherwise specified, all acids were concentrated C.P. reagents.

**REAGENT SOLUTION.** Sodium catechol disulfonate, disodium-1,2-dihydroxybenzene-3,5-disulfonate, was obtained through the courtesy of the Winthrop Chemical Company, Inc., New York, N. Y. This salt is highly soluble in water. Its aqueous solutions are colorless and no evidence of their instability on standing

has been observed. All solutions of the reagent were prepared by dissolving it in distilled water. Some of the solutions were allowed to stand in volumetric flasks for more than 3 months and no difference could be detected between these solutions and freshly prepared ones in either appearance or reactivity with ferric iron.

**STANDARD IRON SOLUTION.** A standard solution of ferric iron was prepared from ferrous ammonium sulfate,  $\text{FeSO}_4(\text{NH}_4)_2\text{SO}_4 \cdot 6\text{H}_2\text{O}$ , of reagent quality. A sample of 7.022 grams of the salt was dissolved in about 100 ml. of distilled water; 5 ml. of sulfuric acid and 1 ml. of bromine were added, and the solution was boiled until all iron was oxidized and excess bromine expelled. This solution containing 1 gram of ferric iron was made up to 1 liter for use as a stock solution.

**SOLUTIONS OF DIVERSE IONS.** The solutions of salts used in studying the effect of various ions on the color reaction were prepared so that each milliliter contained 0.5 mg. of the desired ion. All salts were tested for the presence of iron and if found, either a pure salt was substituted or the contaminated one was recrystallized until iron-free.

**BUFFER SOLUTIONS.** The most satisfactory buffer solutions for color matchings are: sodium acetate-hydrochloric acid mixtures for the blue complex and disodium phosphate for the red complex. The acetate buffer is prepared by dissolving 136 grams of sodium acetate,  $\text{NaC}_2\text{H}_3\text{O}_2 \cdot 3\text{H}_2\text{O}$ , and 66.6 ml. of 12 *N* hydrochloric acid in water and diluting to 2 liters. This solution has a pH of 4.0. The phosphate buffer is prepared by dissolving 71.6 grams of disodium phosphate,  $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ , adding 4 ml. of *N* sodium hydroxide, and diluting to a liter. The pH is about 9.5. A carbonate buffer was also investigated for use with the red form of the iron complex. It is prepared by dissolving 10 grams of sodium hydrogen carbonate and 5 grams of sodium carbonate in water and diluting to 1 liter. The pH is 9.8. The red color produced by the iron complex in the carbonate buffer matches that formed in the phosphate buffer but no particular advantage of one buffer over the other is evident. On long standing, however, the phosphate buffer maintains a more stable color. All buffers must be titanium-free as indicated by a colorless blank with the iron reagent.

## SPECTROPHOTOMETRIC STUDY OF COLOR REACTION

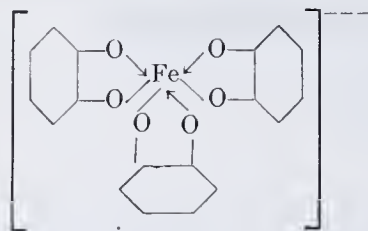
In acid solutions with a pH value below 5, the complex formed by the reaction of disodium-1,2-dihydroxybenzene-3,5-disulfonate and ferric iron is deep blue in color. If the blue solution is made alkaline, the color changes sharply to a violet at pH 5.7 to 6.5 and becomes red at a pH of 7. The exact mechanism of this color change is not understood with certainty. It is believed, however, in the light of evidence obtained in this investigation, that it involves a change in the ratio of the pyrocatechol groups to iron as the hydrogen-ion concentration is changed.

Considerable investigation has been carried out concerning the composition of the iron complex of pyrocatechol, an analog of this reagent. The only difference structurally between pyrocatechol and the new reagent is the presence of two sulfonic acid groups in the 3,5 positions of the latter. It is unlikely that these groups would influence the molecular ratio of combination of the reagent with iron. It has been reported by Reihlen (6), confirmed by

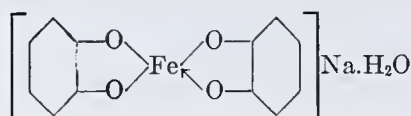
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Weinland and Walter (11), and substantiated by Karrer (3) that the red iron complex of pyrocatechol is a tripyrocatechate ferric anion of the following configuration:



Reihlen also isolated and analyzed a blue salt of ferric pyrocatechol and found it to be as follows:



This configuration corresponds to that found for the antimony analog, fuadin, as determined by Schmidt (8).

From these considerations it would seem that the red and blue forms of the complexes of the new iron reagent are tri- and disodiumsulfonate catechates, respectively.

Experiments were conducted to determine spectrophotometrically whether or not these configurations exist within the colored solutions.

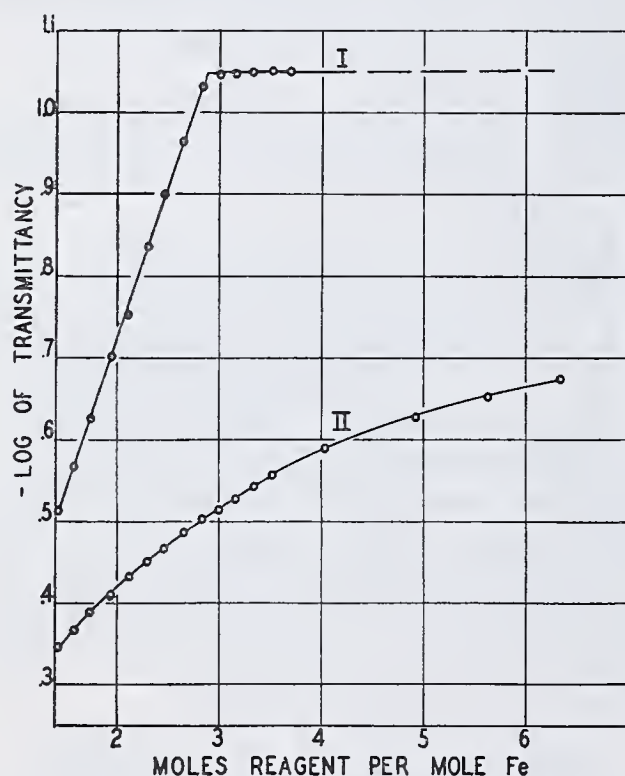


Figure 1. Effect of Ratio of Reagent to Iron

I. Red complex, 480  $m\mu$ , pH 9.5  
II. Blue complex, 620  $m\mu$ , pH 4.0

Two series of solutions were prepared in which the molecular ratio of reagent to iron varied from 1:1 to 7:1. One series was buffered with disodium phosphate at pH 9.5; the other with sodium acetate-hydrochloric acid at 4.0. The absorbency of these solutions was measured spectrophotometrically at the wave lengths of maximum absorbency ( $-\log$  transmittancy) for the two forms, 480 and 620  $m\mu$ , respectively, for the red and blue. The results are presented graphically in Figure 1.

Curve I shows that the color of the red complex does not increase after a 3 to 1 ratio of reagent to iron has been reached. The break in the curve is sudden at this point, indicating that in solution as well as in the solid form the ratio of the sulfonated

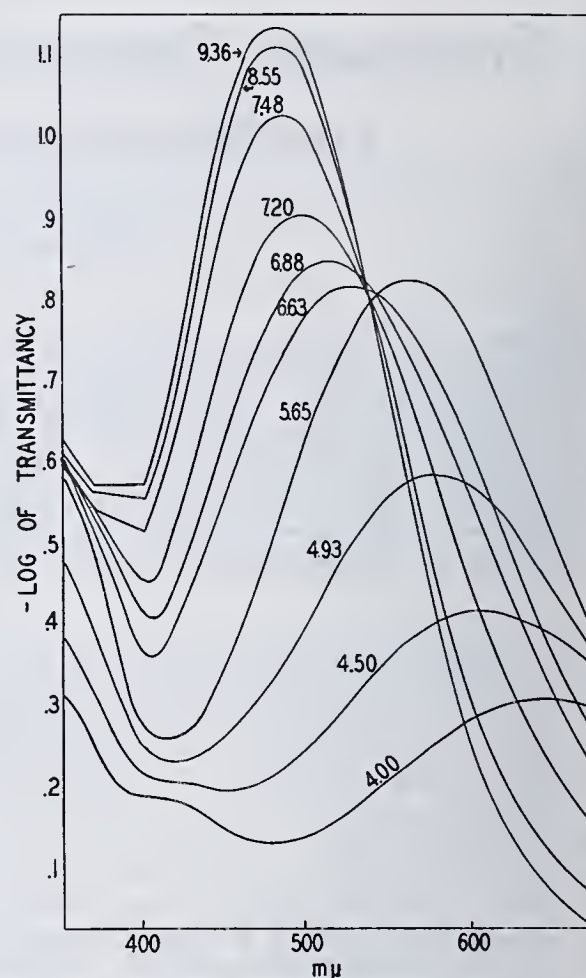
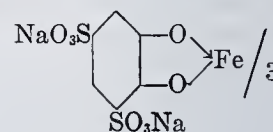


Figure 2. Effect of pH on Iron Complex

pyrocatechol to iron in the complex is 3 to 1 and that it may be represented as follows:



Curve II shows no break but increases in a smooth manner with increasing amounts of reagent. This, however, should not be taken to imply that the blue complex is of indefinite composition in solution but rather that its composition is not indicated by this indirect method of determining it. Since there is no sharp point beyond which increasing amounts of reagent produce no further change in color intensity, the blue complex is less desirable from a colorimetric point of view.

Weinland and Walter (11) isolated the salt of the blue complex of the pyrocatechol analog and found it to have the composition  $[\text{Fe}(\text{OC}_6\text{H}_4\text{O})_2]\text{Na}$ . The blue complex of the new reagent is probably of similar composition, with two moles of sulfonated pyrocatechol combining with one mole of iron to form the complex which behaves as a singly charged anion.

#### SPECTROPHOTOMETRIC STUDY OF EFFECT OF pH ON COLORED COMPLEXES

Variations in hydrogen-ion concentration affect both the intensity and hue of the color produced by the new reagent at pH values below 8.5. Three distinct colors exist within this pH region. Below pH 5.7 the solution is blue, between 5.7 and 7 it is deep violet, and above 7 an intense red. This polychromatic property may be applied in analysis to match aliquot parts of a given sample to a red (pH 9.0 to 10.0), violet (pH 5.7 to 6.5), or blue (pH 3.5 to 4.5) standard by the use of suitable buffers to maintain the pH within the range desired. By utilizing this

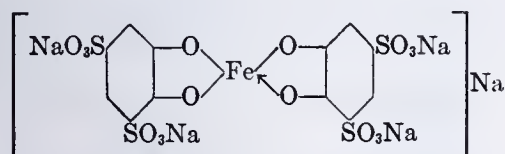


property, errors due to color blindness or color fatigue may be reduced. For instance, if the eyes of the observer are not so sensitive to color differences in the red as they are in the blue or violet, or vice versa, standards may be buffered to the color in which differences are most easily detected.

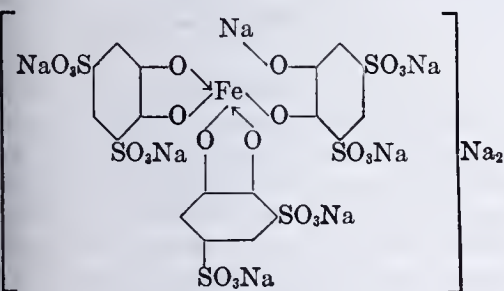
Spectrophotometric measurements were made on a series of solutions containing 10 p.p.m. of iron and varying in pH from 4.00 to 9.36. Absorbency measurements were made at 10 mμ intervals from 350 to 675 mμ. The absorbency (—log of transmittancy) *vs.* wave-length curves are shown in Figure 2. The curves for the solutions of pH 4.00 to 4.93 represent a blue color; from 5.65 to 6.88, violet; and from 7.20 to 9.36, red. The sudden shift in the wave length of the absorbency maxima occurring just above a pH of 5.65 represents the sudden change in color from blue to violet. At an approximate pH of 7 the solution becomes red. The change in the wave length of the maxima of absorbency is represented graphically in Figure 3. Four of the values shown (pH 5.80, 6.08, 6.34, 6.65) were omitted from Figure 2 to avoid congestion.

The abruptness with which these shifts in the position of maximum absorbency occur strongly indicates a definite change in the molecular structure of the colored complexes at the points where the shifts occur. If there were only two complexes and the violet color resulted from a mixture of the red and the blue, one would expect a gradual color transition. But with increasing pH, the blue changes abruptly to violet; the color remains violet over about 2 pH units, and then changes to red. Since the blue compound is believed to be an inner complex singly charged anion and the red compound a triply charged anion, it is possible that between the two, a doubly charged inner complex anion exists with a violet color.

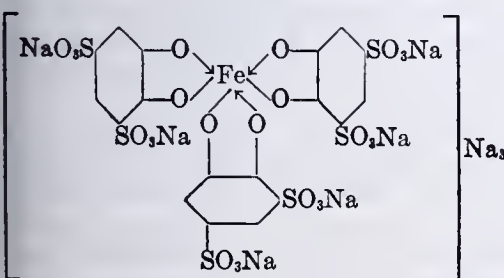
As a result of these spectrophotometric studies it is believed that the three colors, observed with varying pH values, are the result of the formation of three iron-pyrocatechol disulfonate complexes with the following configurations:



Blue Complex



Violet Complex



Red Complex

These studies further show that the new reagent has its greatest sensitivity—i.e., highest value of maximum absorbency—in the red complex. Increases in pH value above 9.5 do not further increase the absorbency or change the position of its maximum with respect to wave length.

The possibility of using the iron complex as a pH or redox indicator has been investigated. It has no value as a redox indicator; however, the change in color with change in hydrogen-ion concentration suggests its use as a pH indicator. With increasing pH, there is a sharp change from blue to violet at pH 5.7; but the change from violet to red is not sufficiently sharp for end-point determination in acid-base titrations. Mixtures of carbonates and bicarbonates may be analyzed by using the complex in place of a double indicator, but the results are only roughly quantitative.

#### BEER'S LAW APPLIED TO NEW IRON COMPLEXES

Beer's law is valid for both the red and blue complexes at the wave lengths of maximum absorbency. The violet complex was not tried, owing to failure to find a satisfactory buffer for it. [Spectrophotometric measurements were made using 1-cm. transmission cells over a concentration range of 0.2 to 10 p.p.m. of iron. Neither the red nor the blue complex shows any deviation from Beer's law within this range.]

#### SENSITIVITY OF REACTION

The limit of sensitivity of the red complex is the detection of 1 part of iron in 200,000,000 parts of solution, when observation is made in either 50-ml. tall-form Nessler cylinders or the Yoe roulette comparator. Most colorimetric reagents for ferric iron have sensitivity limits in the range of 1 part in 10,000,000 to 30,000,000 under the most favorable conditions. The limit of sensitivity was established in the following way:

Solutions containing 1 part of iron in 50,000,000, 100,000,000, and 200,000,000 parts of solution, respectively, were prepared by adding 0.5 ml. of reagent solution (0.113 gram per 100 ml.) to 2.00, 1.00, and 0.50 ml. of a solution containing 0.001 mg. of iron per ml. and diluting to a total volume of 100 ml. with a sodium carbonate-bicarbonate buffer of pH 9.5. In Nessler cylinders it was possible to determine correctly the respective order of concentration and definitely distinguish the solution containing 1 part of iron in 200,000,000 from a blank.

The limiting sensitivity of the blue complex was determined in the same manner and found to be 1 part of iron in 30,000,000

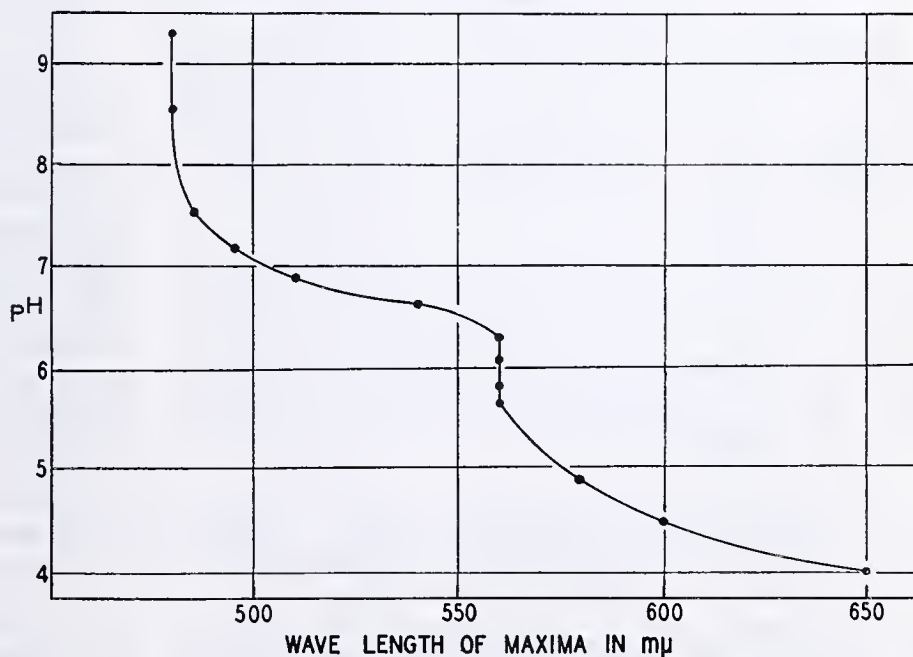


Figure 3. Effect of pH on Wave Length of —Log of Transmittancy Maxima



parts of solution. When potassium hydrogen phthalate is used as a buffer (pH 4.0), the sensitivity is decreased to 1 part in 20,000,000.

Optimum concentrations for visual study of the red complex are within the range of 0.05 to 2 p.p.m. of iron. The region of greatest sensitivity between fixed small increments is in the range of 0.08 to 0.12 p.p.m. of iron. Within this range, solutions differing from each other by 1 part of iron in 100,000,000 may be identified with certainty. At concentrations in the neighborhood of 1 p.p.m., the color intensity of the red complex prevents detection of differences greater than 1 part in 50,000,000.

Increments differing by 1 p.p.m. of iron may be detected by the blue complex. Since the intensity of the blue is less than that of the red, the optimum range for the detection of small increments lies between 0.6 and 1 p.p.m. The sensitivity is greater with sodium acetate-hydrochloric acid buffers than with potassium hydrogen phthalate.

#### SPOT-PLATE SENSITIVITY

Spot-plate sensitivity tests were made by transferring 0.05 ml. of standard iron solution to a depression in a white porcelain spot plate, adding 0.05 ml. of reagent solution (0.0036 *M*) and finally 0.05 ml. of buffer solution. Tests were made with both the red and blue complexes by using disodium phosphate and sodium acetate-hydrochloric acid buffers, respectively. Blanks were prepared by adding buffer solutions to the reagent alone.

One drop of solutions containing 1 p.p.m. of iron gave tests which could be distinguished from blanks with certainty, for both the red and blue complexes. This represents the detection of 0.05 microgram of iron. The limit of sensitivity on the spot plate is the same for either complex, red or blue.

#### PERMANENCY OF STANDARDS

The color intensity of both the red and blue complexes increases slightly for the first 18 hours (about 5% transmittancy at the wave lengths of maximum absorbency), after which time it remains constant for many months. Solutions of the colored complexes have been kept in stoppered tubes in the diffuse light of the laboratory for 2 years. These solutions showed no change in intensity when compared visually with freshly prepared solutions that stood for 18 hours. Since there is a slight change in the intensity during the first 18 hours, it is best to prepare fresh standards daily for precise work. The preparation of standards is so simple that artificial color standards offer little or no advantage.

#### EFFECT OF DIVERSE IONS

REACTIONS WITH VARIOUS IONS. Tests were made on spot plates by adding a drop of reagent to a drop of the solution containing the respective ions (approximately 0.05 mg.). Acid, alkaline, and neutral media were used where feasible. No color or precipitate formation was observed with any of the following 69 ions:

Al<sup>+++</sup>, As<sup>+++</sup>, AsO<sub>4</sub><sup>---</sup>, B<sub>4</sub>O<sub>7</sub><sup>---</sup>, Ba<sup>++</sup>, Be<sup>++</sup>, Bi<sup>+++</sup>, Br<sup>-</sup>, CO<sub>3</sub><sup>---</sup>, Ca<sup>++</sup>, CbO<sub>4</sub><sup>---</sup>, Cd<sup>++</sup>, Ce<sup>+++</sup>, Cl<sup>-</sup>, Co<sup>++</sup>, Cr<sup>+++</sup>, Cs<sup>+</sup>, Dy<sup>+++</sup>, Er<sup>+++</sup>, Eu<sup>+++</sup>, F<sup>-</sup>, Fe<sup>++</sup>, Ga<sup>+++</sup>, Gd<sup>+++</sup>, Ge<sup>+++</sup>, HfO<sup>++</sup>, Hg<sup>+</sup>, Hg<sup>++</sup>, I<sup>-</sup>, In<sup>+++</sup>, Ir<sup>+++</sup>, K<sup>+</sup>, La<sup>+++</sup>, Li<sup>+</sup>, Mg<sup>++</sup>, Mn<sup>++</sup>, NO<sub>2</sub><sup>-</sup>, NO<sub>3</sub><sup>-</sup>, Na<sup>+</sup>, Nd<sup>+++</sup>, HPO<sub>4</sub><sup>---</sup>, Pb<sup>++</sup>, Pd<sup>++</sup>, Pr<sup>+++</sup>, PtCl<sub>6</sub><sup>---</sup>, Rb<sup>+</sup>, ReO<sub>4</sub><sup>-</sup>, Rh<sup>+++</sup>, Ru<sup>+++</sup>, S<sup>-</sup>, SO<sub>4</sub><sup>---</sup>, Sb<sup>+++</sup>, Sc<sup>+++</sup>, SeO<sub>3</sub><sup>---</sup>, SiO<sub>3</sub><sup>---</sup>, Sm<sup>+++</sup>, Sn<sup>++</sup>, Sn<sup>++++</sup>, Sr<sup>++</sup>, TaO<sub>4</sub><sup>---</sup>, TeO<sub>4</sub><sup>---</sup>, Th<sup>++++</sup>, Ti<sup>+++</sup>, Tm<sup>+++</sup>, WO<sub>4</sub><sup>-</sup>, Y<sup>+++</sup>, Yb<sup>+++</sup>, Zn<sup>++</sup>, ZrO<sup>++</sup>.

The following ions produce colored solutions with the reagent: Fe<sup>+++</sup> (blue or red depending on the pH), MoO<sub>4</sub><sup>---</sup> (yellow), OsO<sub>3</sub><sup>---</sup> (yellow), Cu<sup>++</sup> (greenish yellow in alkaline solution), UO<sub>2</sub><sup>++</sup> (yellow), VO<sup>+</sup> (purple color which fades to colorless within 15 minutes), and Ti<sup>++++</sup> (an intense yellow solution). Ag<sup>+</sup> and AuCl<sub>4</sub><sup>-</sup> are reduced by the reagent to the metal. A total of 78 ions was tested but only 7 produce colored solutions;

of these, the reaction with titanium is the most intense. Ferric iron is the only element which produces either a blue or red solution under any conditions.

DETERMINATION OF IRON IN THE PRESENCE OF OTHER IONS. In measuring the effect of diverse ions, a standard solution of the ion in question was added to 0.05 mg. of iron, 1 ml. of reagent solution (0.0075 *M*) introduced, the solution diluted to 50 ml. (Nessler cylinder, tall-form) with a buffer, and visual observations were made to match the solution with a standard of the same concentration of iron. The results are recorded in Table I.

Table I. Effect of Diverse Ions

Ion	Added as	Amount Present	Remarks
		P.p.m.	
Al <sup>+++</sup>	Al(NO <sub>3</sub> ) <sub>3</sub>	> 5	RX, BX
		< 5	R, B
Be <sup>++</sup>	BeCl <sub>2</sub>	> 100	R, B
B <sub>4</sub> O <sub>7</sub> <sup>---</sup>	Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub>	> 0.5	R, BX
Ca <sup>++</sup>	Ca(NO <sub>3</sub> ) <sub>2</sub>	> 10	R (if pptd. Ca i filtered), B
		< 10	R, B
Co <sup>++</sup>	Co(NO <sub>3</sub> ) <sub>2</sub>	> 25	RX, B
		< 25	R, B
Cr <sup>++</sup>	Cr(NO <sub>3</sub> ) <sub>3</sub>	> 10	RX, BX
		< 10	R, B (if done quickly)
Cr <sub>2</sub> O <sub>7</sub> <sup>---</sup>	K <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub>	> 1	RX, BX
		< 1	RX, B
Cu <sup>++</sup>	Cu(NO <sub>3</sub> ) <sub>2</sub>	> 1	RX, BX
		< 1	R, B
F <sup>-</sup>	KF	> 100	R, BX
		< 100	R, BX
Fe <sup>++</sup>	FeSO <sub>4</sub> (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	> 50	R, B
Mg <sup>++</sup>	MgSO <sub>4</sub>	> 50	R (if pptd. Mg i filtered), B
		< 50	R, B
Mn <sup>++</sup>	MnCl <sub>2</sub>	> 100	R (if pptd. Mn i filtered), B
		< 100	R, B (if done quickly)
NH <sub>4</sub> <sup>+</sup>	NH <sub>4</sub> Cl	> 1000	RX, BX
		< 1000	R, B
Ni <sup>++</sup>	Ni(NO <sub>3</sub> ) <sub>2</sub>	> 25	RX, BX
		< 25	RX, B
		< 10	R, B
PO <sub>4</sub> <sup>---</sup>	Na <sub>3</sub> PO <sub>4</sub>	> 100	R, B
SiO <sub>3</sub> <sup>---</sup>	Na <sub>2</sub> SiO <sub>3</sub>	> 100	R, B
Sn <sup>++</sup>	SnCl <sub>2</sub>	> 1	RX, BX
		< 1	R, B
Sn <sup>++++</sup>	SnCl <sub>4</sub>	> 1	RX, BX
		< 1	R, B
Ti <sup>++++</sup>	TiCl <sub>4</sub>	> 0.01	RX, B
ZrO <sup>++</sup>	Zr(NO <sub>3</sub> ) <sub>4</sub>	> 5	R, B
Phthalate	Potassium hydrogen phthalate	> 5	R, B
Citrate	Citric acid	> 5	R, BX
Maleic	Maleic acid	> 5	R, B
Oxalate	Oxalic acid	> 5	R, BX
Tartrate	Tartaric acid	> 5	R, BX

Key to symbols: R = red matches, B = blue matches, RX = red does not match, BX = blue does not match.

These studies show that there are relatively few ions which cause interference in the color reaction and none which cannot be removed by ordinary procedures of analysis. Of the several ions which form colored solutions with the reagent, only two or three—titanium, copper, and possibly molybdenum—are likely to be present in materials in which iron is determined colorimetrically. All are easily separated from iron by means of hydrogen sulfide. In substances such as glasses, glass sands, rocks and clays, titanium is apt to be present with iron. If the amount of titanium is known, iron may be determined in its presence simply by adding the corresponding quantity of titanium to the standards. If the titanium content is not known, it may be separated from iron by precipitating the latter with ammonium sulfide in the presence of tartrate (1).

The color intensity of the titanium complex is very great (bright lemon yellow) and should be useful for the colorimetric determination of titanium. Preliminary experiments indicate that the sensitivity of the titanium reaction is about 1 part of titanium in 200,000,000 parts of solution. Apparently the color is not affected by changes in pH. A complete study of this reaction is in progress.



## OPTIMUM EXPERIMENTAL CONDITIONS

The use of disodium-1,2-dihydroxybenzene-3,5-disulfonate in the colorimetric determination of iron requires no unusual departure from standard colorimetric procedures. The reagent has the important advantage of being colorless and highly soluble in water. Excess of the reagent produces no change in the color of the complex. By utilizing the red complex, which is completely stable in alkaline solution, iron may be determined in the presence of large amounts of fluoride, tartrate, oxalate, citrate, phosphate. This is impossible with most colorimetric reagents for iron. The interference encountered with some ions, such as antimony and copper, is typical for the general class of *o*-dihydroxybenzene and *o*-hydroxycarboxybenzene colorimetric iron reagents.

Table II. Determination of Iron

Sample	Material	Fe <sub>2</sub> O <sub>3</sub> , N.B.S. Value	Fe <sub>2</sub> O <sub>3</sub> Found	Difference
		%	%	%
1	N.B.S. feldspar, No. 70	0.03	0.04	+0.01
2			0.04	+0.01
3	N.B.S. fluorspar, No. 79	0.15	0.16	+0.01
4			0.15	0.00
5	N.B.S. glass sand, No. 81	0.073	0.073 <sup>a</sup>	0.000
6			0.076 <sup>a</sup>	+0.003
7	N.B.S. dolomite, No. 88	0.084	0.087	+0.003
8			0.085	+0.001
9	N.B.S. silica brick, No. 102	0.66	0.70	+0.04
10			0.70	+0.04
11	N.B.S. soda-lime glass, No. 128	0.039	0.036	-0.003
12			0.035	-0.004
13			0.036	-0.003
14			0.038	-0.001
		Fe, N.B.S. Value	Fe Found	
		%	%	
15	N.B.S. sheet brass, No. 37b	0.21	0.16 <sup>b</sup>	-0.05
16			0.15 <sup>b</sup>	-0.06
17			0.15 <sup>c</sup>	-0.06
18			0.16 <sup>c</sup>	-0.05
19			0.17 <sup>c</sup>	-0.04

<sup>a</sup> Added to standards an amount of TiO<sub>2</sub> corresponding to 0.10%.

<sup>b</sup> Tin separated as metastannic acid; iron doubly precipitated as hydroxide and redissolved in hydrochloric acid.

<sup>c</sup> 1-gram samples dissolved in aqua regia; other constituents separated from iron as sulfides.

**RECOMMENDED PROCEDURE OF ANALYSIS.** After a representative portion of the material to be analyzed has been procured and given the necessary preparative treatment, weigh out a sample containing 1 mg. of iron or less. The sample should not be larger than this because 1 mg. of iron is sufficient to prepare 1 ml. of solution containing 1 p.p.m. When using 50-ml. tall-form Nessler cylinders or the Yoe roulette comparator, the range of maximum sensitivity is between 0.1 and 1 p.p.m. Hence, whenever possible the sample size should be selected so that convenient aliquot parts of its solution will permit an iron concentration within this range.

Dissolve the sample by an appropriate method using either fusions, treatments with mineral acids, or both. Remove interfering ions (if present in amounts greater than tolerances found in Table I) by the usual methods of separation. Transfer the solution containing iron to a 100-ml. volumetric flask and dilute with water. Mix thoroughly and transfer a 5-ml. aliquot to a Nessler cylinder. Add 1 ml. of reagent solution (0.0075 *M*, molecular weight 358.2) and dilute to the mark with disodium phosphate buffer (pH 9.5) or sodium acetate-hydrochloric acid (pH 4.0), depending on whether the red or the blue complex is to be matched. After thorough mixing, compare the colored complex of the sample with standards buffered to the same pH and covering the range of concentration under consideration.

To prepare standards, introduce standard iron solutions to Nessler cylinders, then add the reagent, mix thoroughly, dilute to the mark with the buffer solution and again mix.

## DETERMINATION OF IRON IN VARIOUS MATERIALS

The applicability of the new reagent to the colorimetric determination of iron in various materials was tested by analyzing a

representative group of National Bureau of Standards samples (Table II).

## DISCUSSION OF RESULTS

The results in Table II show that the reagent may be applied successfully to the colorimetric determination of small amounts of iron in various materials. With the exception of N.B.S. sheet brass No. 37b, all the values determined by the new reagent are well within the range of values reported by the bureau. The accuracy of the method is equal to that of any of the methods used in determining the values reported by the bureau. The precision between individual analyses is excellent. The value found for sheet brass No. 37b is lower than that reported by the bureau by an average of 0.05%. This sample was analyzed colorimetrically by Yoe and Hall (13) using 7-iodo-8-hydroxyquinoline-5-sulfonic acid (ferron). Their values were 0.18 and 0.18% iron on two analyses as compared with an average value of 0.16% iron found by means of the new reagent. It is believed that these low results are due to the inadequacy of the methods employed in separating the iron rather than to any inability of the reagent to react quantitatively with the iron actually present.

In the presence of strong oxidizing agents, the reagent may be darkened as a result of its oxidation. For example, perchloric acid solutions bring about a slight darkening of the reagent which causes difficulty in matching. In an analysis of N.B.S. soda-lime glass No. 128 perchloric acid was used to dehydrate the silica. All the perchloric acid was not removed before the addition of the reagent and as a result the samples were slightly darkened. Matchings made with these solutions gave values which were on an average 0.015% higher than the average value of the bureau. In the absence of perchloric acid, the values obtained with the new reagent agreed with the bureau's average value within a few thousandths per cent.

Precise matchings are more easily made in alkaline medium with the red complex using disodium phosphate as a buffer. Once formed, the iron complex will prevent the precipitation of hydrated ferric oxide regardless of the pH value. If elements such as calcium, magnesium, and aluminum are present, they may precipitate when the solution is made alkaline. This presents no serious difficulty; any precipitate is filtered off before comparison with the standards. With the materials reported in Table II, filtration was necessary in only two instances (Nos. 70 and 88).

In general, the procedure to be used in analyzing most materials is very simple. Sodium carbonate fusions dissolved in dilute hydrochloric acid and filtered directly into a volumetric flask will provide a solution from which aliquot parts may be taken for immediate matching upon addition of the reagent and buffer solution. Dehydration of silicic acid in the fusion solution is not necessary.

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# Thiamine in Beef Muscles

## A Comparison of Values by the Thiochrome Reaction Applied with and without Adsorption

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The Harris and Wang nonadsorption thiochrome technique and the Hennessy and Cerecedo base-exchange technique have been compared, as applied to beef muscle. Although 40 to 100% discrepancies were frequently found, only up to 20% differences between the two values could be explained by incomplete adsorption, resulting in low Hennessy values when 2 to 5 micrograms of beef thiamine were placed on a column. Comments are made on enzyme digestions and on the Harris thiochrome reaction. A few comparisons with yeast fermentation values are also included.

IN THE first comparisons of the applications of the Harris and Wang (14, 34) and the Hennessy and Cerecedo (15, 30) thiochrome techniques to identical digests of beef muscle, the authors found the values obtained from the former exceeding those from the latter by 40 to 100%. These discrepancies raised the questions as to whether the direct oxidation technique applied to beef results in the inclusion of nonthiochrome fluorescent compounds which would be eliminated in the Hennessy base-exchange technique and whether the latter procedure can be depended upon to adsorb all the thiamine from beef digests.

The data here presented indicate that in some samples of beef, nonthiochrome substances do interfere in direct oxidation, but that, on the other hand, the adsorption procedure is likely to give results which are around 10 to 20% too low if an amount of beef digest containing from 2 to 5 micrograms of thiamine is placed on a 7- to 8-cm. Decalso column. Although no data were obtained on other foods, this experience with beef would indicate a need for caution in the indiscriminate application of the Harris and Wang technique to low thiamine foods as well as in the application of the Hennessy and Cerecedo technique to such foods when this is done in the same quantitative relation of thiamine to Decalso that has been described as optimal for cereals (30).

Nonadsorption methods of thiochrome determination in both animal and vegetable tissues, which vary in details of digestion, of the final oxidation reaction, and of the reading of the fluorescence, have been described in a few American reports (2, 16, 22) and in an earlier German one (36), as well as in many British reports (7, 14, 19, 26-29, 34). On the other hand, applications of the Hennessy and Cerecedo base-exchange technique have been widely reported in this country (1, 2, 9, 10, 18, 21, 25) and a similar adsorption procedure was described in one earlier Dutch study (35).

Figures on beef muscle are included in only one paper from each of the two groups listed above (18, 29), and all their figures are in the range covered by the authors' Hennessy values. Lane, Johnson, and Williams (18) reported verification of their thiochrome results on beef by the curative bioassay. Further comparison of the authors' results with other recent bioassay reports on beef showed that the Hennessy value on their first sample of round—namely, 1.0 to 1.1 micrograms per gram—was in good agreement with one figure on lean top round obtained by Booher (6) by a rat-growth method, 1.14 micrograms per gram whereas their Harris value on this round, though 40 to 50% higher than the Hennessy, was just at the lower end of the range of values obtained on raw round by Waisman and Elvehjem (33) with the rat-growth method of Arnold and Elvehjem (3). The authors' first Hennessy values on rib ranged between 0.6 and 0.8 microgram per gram and Harris values between 1.2 and 1.3 micrograms per gram. Yet they learned that McLaren and Cover (23), using papain-takadiastase digests, were getting Harris values on beef rib which varied between 1.9 and 2.8 micrograms per gram, most of them in the upper end of this range and thus close to the highest value reported for raw round by Waisman and Elvehjem, 2.8 micrograms per gram.

Older reports based on bioassay methods include more figures on beef in the lower range—bradycardia method (5), Sherman Chase growth method (8), Cowgill calculation from pigeon protective and rat-growth data (12), rat-growth method calculated as Sherman units by Daniel and Munsell (13, 31)—than in the higher range—rat-curative method, one value, calculated as Sherman units by Daniel and Munsell (13, 17); bradycardia method, one figure, highest, stated to have probable error of 50% (4, 5).

Thus, with bioassay values in the literature falling in ranges which are 100% apart, it was not possible to find support for either of the thiochrome techniques exclusively.

### DISCUSSION OF TECHNIQUES

No important modifications were made in most of the details of the assay methods and for this reason the exact procedure is not given here. In the Hennessy method the authors combine details from three sources (10, 24, 30) and in the Harris method they used essentially the revised Wang and Harris procedure (34). However, it seems pertinent to discuss a few points in the techniques.

**SAMPLING AND DIGESTION.** There has been some general opinion among authorities that the complete extraction of thiamine from tissues is difficult (25). For this reason, the authors felt it desirable with ground beef to keep the sample solvent ratio down to the middle range recommended for cereals—that is, about 1 to 25 or 1 to 20 during acid extraction. They note, from some later experience with more concentrated digests of beef samples blended in the Waring Blendor, that a concentration of 1 to 10 or even 1 to 8, sample to solvent, can be safely employed with motor stirring during extraction at 70° to 75°. Digestion with clarex was carried out overnight at 45° to 48°.

In view of the fact that digestion was carried out and volume made up in calibrated 250-ml. centrifuge bottles it was found convenient to clear digests by centrifuging after vigorous shaking with 5 ml. of reagent grade chloroform which took the fat to the bottom. That the chloroform had no effect on the adsorption of thiamine (or riboflavin, 11) or its subsequent determination of fluorescence was shown by comparison of values with filter portions of identical digests not exposed to chloroform.

**HENNESSY BASE-EXCHANGE.** Before being used in this study all samples of Decalso had been tested by placing 5 micrograms of buffered synthetic thiamine hydrochloride on 7- to 8-cm. columns. Recoveries were always 95% or better with the adsorption and elution techniques employed. All adsorptions and elutions were carried out at room temperature. However, in the spring of 1943 samples of Decalso were obtained from which complete elution could be accomplished with 25 ml. of 25% potassium chloride-0.1 *N* hydrochloric acid only if the latter was used at practically boiling temperature. The experiment reported in Table V was done with such a sample of Decalso.

**HARRIS THIOCHROME REACTION.** Harris (14) states that excess of ferricyanide is to be avoided to prevent a partial destruction of the thiochrome formed and variable effects on nonspecific fluorescent substances. In attempting to determine the correct amount of ferricyanide to add, by applying the Wang and Harris (34) criterion of "yellow color lasting more than 30 seconds" some difficulty was encountered because of the faint brownish yellow color of the digests which was accentuated by the preliminary addition of alkali and because additional drops of ferricyanide progressively increased the readings. (Throughout the study the amounts of 2% potassium ferricyanide used per action were 0.03 to 0.08 ml., varying from 1 to 3 drops depending on the size of drop and on the digest.) Frequently, one additional drop used when the excess was questionable increased the "thiochrome" value as much as 10%. Since the authors have never observed a decrease in readings with larger excesses of ferricyanide, even though thiochrome itself is known to be unstable under these conditions, it would seem as though beef digests con-



in some material, other than thiamine, which can add to the reading with this direct oxidative treatment. Such a possibility exists some support in the observation that very large excesses of ferricyanide, such as 2 ml. of 2% potassium ferricyanide, cause increases in fluorescence up to 40% of the thiochrome value as determined by the usual Wang and Harris criterion.

**STANDARDIZATION.** For all the Harris determinations and for the first group of Hennessy experiments (1 through 5, Table I) standardization was done with "external standards"—that is, crystalline thiamine in proper diluent, reacted by itself. Later Hennessy experiments (6 through 9) were based on reading of crystalline thiamine added to the unknown eluates, "internal standards" (24). Although occasional internal standards read as high or higher than "external" ones the average internal standard readings were about 6% lower than the average of external standards reacted in this series. Hence, the Hennessy values of the first group of experiments (samples 1 through 5, Table I) could be corrected upward by about 6% to be on the same basis as the second group.

Fluorescence readings of "external standards" over several months averaged: for Hennessy, using 15 ml. of isobutanol,  $10.8 \pm 2.2$  galvanometer units per 0.1 microgram; for Harris, using 10 ml. of methanol, 10 ml. of isobutanol, and 2 ml. of ethanol,  $1.35 \pm 0.37$  units per 0.1 microgram. For both, a quinine working standard containing 0.0135 mg. of quinine sulfate per 100 ml. 0.1 N sulfuric acid was set at 60 on the Coleman electronic photofluorometer (Model 12).

### DISCUSSION OF RESULTS

No doubt, pronounced differences in thiamine content of various beef samples are to some extent responsible for the variations found in reported values and for the variations in values obtained by the Harris thiochrome technique in the authors' laboratory and the Texas laboratory. Moreover, if the Harris technique measures the additional fluorescent material produced in the oxidation reaction, besides thiochrome, variations in the occurrence of each other factor would also contribute to differences between samples analyzed in the two laboratories by this method.

The authors sought to raise their values on beef by digesting with papain because Harris stated that papain removes an inhibition (present in animal tissues) to hydrolysis of cocarboxylase or takadiastase. If this is true, papain digests ought to show higher thiochrome values than acid-clarase digests by both the

Harris and Hennessy methods, but as results recorded in Table I indicate, the authors have not once obtained higher values by either Harris or Hennessy thiochrome technique on papain-clarase or papain-takadiastase digests. However, they cannot dismiss the possibility of an influence on Harris values related to the method of digestion because McLaren, working in the authors' laboratory, made four papain-clarase digests of rib sample 5 (Table I) on which they obtained an average Harris thiochrome value of 1.54 micrograms per gram as contrasted to the 1.15 micrograms per gram (see table) for the acid clarase digests made on the same day, whereas the Hennessy values were in agreement for the two. The only possible fact to which this variation in Harris values can be ascribed is the use of a different papain, for McLaren used a Difco preparation, whereas the authors have used Parke, Davis preparations from two different lots, both of which have shown strong proteolytic activity in that they dissolved the beef almost completely. It is possible that the higher Harris thiochrome results are related to the presence in some papain samples of another enzymatic activity other than the proteolytic. The authors hope to investigate this further.

According to McIntire and Elvehjem (20) the thiochrome values on pork muscle are the same whether digestion is done with papain-clarase or acid-clarase. Moreover, in the acid-clarase digests, the adsorption and the direct oxidation techniques give the same thiochrome value.

In attempting to discover the reason for the difference in values with and without adsorption the first line of attack used was one suggested by Dr. Elvehjem—applying sulfite blanks to the Harris values obtained on the beef digests. The sulfite treatment done at room temperature overnight at pH 5 to 5.5 (37) was more satisfactory than the short treatment at boiling temperature (32) because with the latter new fluorescent material was formed which added to the reading after ferricyanide treatment. However, the oxidized fluorescence values left after sulfiting at room temperature were variable, sometimes so low that if applied on the Harris values as blanks, they would leave thiamine values higher than the Hennessy by 10 to 40%, whereas in one test the residual values were so large as to reduce the Harris values far

Table I. Thiamine Values on Beef

(Showing variation in discrepancies between Harris and Hennessy thiochrome results)

Sample No.	Cut and Grade	Length of Aging Period Days	Fresh or Frozen Storage Period	No. and Type of Digests	Volume Adsorbed <sup>a</sup> ML.	Harris Thiochrome	Thiochrome	Hennnessy Thiochrome	Difference between Harris and Hennnessy Values	
						On digests γ/g.	On filtrates from adsorption γ/g.	on Eluates γ/g.	Percentage of Hennnessy value	
1	Rib, packer's grade 4	21	Raw frozen 96 days	4, H <sub>2</sub> SO <sub>4</sub> clarase	100	1.29	0.67	0.64	0.65	101
				4, papain clarase	100	1.30	0.72	0.61	0.69	113
	Rib, packer's grade 4 (heifer)	8	Raw frozen 72 days	2, H <sub>2</sub> SO <sub>4</sub> clarase	50	1.22	..	0.78	0.44	56
3	Whole round, packer's grade 3 (heifer)	11	Raw fresh	4, papain clarase	80	1.48	0.62	0.97	0.51	53
			Raw frozen 21 days	6, H <sub>2</sub> SO <sub>4</sub> clarase	100	1.55	..	1.03	0.52	50
			Raw frozen 34 days	5, H <sub>2</sub> SO <sub>4</sub> clarase	75	1.55 <sup>b</sup>	..	1.11 <sup>b</sup>	0.44	40
4	Chuck, packer's grade 3 (steer)	4	Raw fresh	3, H <sub>2</sub> SO <sub>4</sub> clarase	75	0.82	0.34	0.49	0.33	67
			Cooked fresh	3, H <sub>2</sub> SO <sub>4</sub> clarase	75	0.66	0.26	0.45	0.21	47
5	Rib eye	?	Raw fresh	3, H <sub>2</sub> SO <sub>4</sub> clarase	70	1.15	..	0.58	0.57	98
6	Outside round, U. S. grade commercial (steer)	5	Raw frozen 7 days	4, H <sub>2</sub> SO <sub>4</sub> clarase	100	1.03	0.48	0.69	0.34	49
7	Rib, U. S. grade good	9	Raw fresh	4, H <sub>2</sub> SO <sub>4</sub> clarase	100	1.03	0.44	0.77	0.26	34
			Raw frozen 41 days	2, H <sub>2</sub> SO <sub>4</sub> clarase	100	1.19	0.36	0.90	0.29	24
				2, papain takadiastase	100	1.04	0.42	0.85	0.19	22
			Raw frozen 74 days	2, H <sub>2</sub> SO <sub>4</sub> clarase	50	1.16	..	0.91	0.25	21
8	Outside round, U. S. grade commercial (heifer)	6	Raw fresh	3, H <sub>2</sub> SO <sub>4</sub> clarase	100	1.29	0.32	1.15	0.14	12
				1, HCl takadiastase	25*	1.19	0.04	1.22	-0.03	0
			Raw frozen 45 days	2, H <sub>2</sub> SO <sub>4</sub> clarase	25	1.34	..	1.19	0.15	13
					50					
9	Liver	?	Raw fresh		75					
				1, H <sub>2</sub> SO <sub>4</sub> clarase	35	2.88	..	2.55	0.33	14
				1, papain clarase	35	2.62	..	2.70	-0.08	0
				1, HCl takadiastase	20*	2.82	..	2.93	-0.07	0

<sup>a</sup> Beef concentrations were between 0.035 and 0.040 gram per ml. in all digests except starred one of sample 8, 0.133 gram per ml. and starred one of sample 9, 0.061 gram per ml.

<sup>b</sup> Crystalline thiamine added to two of the five digests included in these averages was equivalent to 0.71 γ and 0.63 γ per gram of sample and the recoveries based on the sample averages here given were by Harris 97 and 98%, respectively, and by Hennessy 100 and 87%, respectively.



Table II. Fluorescence by Harris Thiochrome Technique on Sulfited Beef Digests<sup>a</sup>

Digest No.	Sample 3, 7/28/42 Digests		Sample 4, 8/25/42 Digests		Sample 7b, 1/10/43 Digests	
	Sulfited	Sulfited	Raw	Cooked	Sulfited	Sulfited
	7/31	8/3	8/28	8/28	1/18	1/27
	$\gamma/g.$	$\gamma/g.$	$\gamma/g.$	$\gamma/g.$	$\gamma/g.$	$\gamma/g.$
1	0.21	0.23	0.20	0.08	0.19	..
2	0.19	0.33	0.15	0.16	0.23	0.37
3	0.00	0.36	0.26	0.24	0.00	0.65
4	0.18	0.33	..	..	0.34	0.60
5	0.09	0.25	..	..	..	..
6	0.13	0.21	..	..	..	..
Av.	0.13	0.28	0.20	0.16	0.25	0.54
Harris minus sulfite blank	1.42	1.27	0.62	0.50	0.87	0.58
Hennessy	1.03	0.49	0.45	0.88		

<sup>a</sup> Calculated as  $\gamma$  of thiamine per gram of beef.<sup>b</sup> Digests 1 and 2 were acid clarase, 3 and 4 were papain-clarase.

below the Hennessy. The erratic results with repeated sulfite treatments of the same digests on different days and the variability between the several digests of one sample are shown in Table II, which repeats some Harris and Hennessy values given in Table I. Because there is such variation the authors felt insecure in assigning too great significance to sulfite for destruction of thiamine only, nor the prevention of formation of a new interfering oxidized fluorescence could be depended upon when measuring such fluorescence in beef digests.

Since Wang and Harris (34), following McFarlane (19), suggest the use of hydrogen peroxide after the ferricyanide treatment to destroy interfering fluorescent substances in urines, milk, and yeast extracts, the authors made a few comparative tests on beef digests with 5 drops of 5% hydrogen peroxide per test as Harris uses it and also with 1 ml. of 30% hydrogen peroxide as McFarlane and Chapman direct for extracts of grass, wheat germ, yeast, and flour. As Table III indicates, peroxide treatment did not destroy any of the fluorescence present after oxidation of a beef-digest aliquot.

Since the difference between the two thiochrome values on the same digest of beef muscle is more than equaled by the values obtained when applying the Harris thiochrome technique to filtrates from adsorption (Table I), the determination of whether or not such filtrations contain any appreciable thiamine activity seemed to be the best approach to the problem. Using such filtrates for rat assay was suggested by R. R. Williams.

The completion of a conclusive experiment of this kind was frustrated by the fact that three samples of beef bought in large quantity for this purpose (samples 6, 7, and 8) showed less actual difference, expressed as micrograms per gram, in the thiamine values obtained by the two methods of assay than most earlier samples. In fact, no real difference beyond experimental error was found between the two values in sample 8 and in a beef liver sample, No. 9, which was tested with the thought that this tissue might display more discrepancy between the two techniques. Such low differences brought about problems in the preparation of concentrated filtrate to be fed to rats. The authors felt that to be tested for activity the filtrate fed each day must represent at least 1 microgram of thiamine difference between the two thiochrome values. For sample 7, with which a limited rat experiment was carried out, this meant that filtrates from adsorption needed to be concentrated so that 1.2 ml. of final preparation represented 4 grams of the original beef. The concentrate was tube-fed to 20 rats in four groups, as shown by the chart of average weight changes (Figure 1). Although the results cannot be considered as conclusive because of the small number of animals used, they indicated that there was a slight thiamine activity in the filtrate from the adsorption. This was further substantiated by the fact that, whereas three out of five of the negative control animals, Group D, died on the 17th, 18th, and 20th day of the supplementing period, among five animals on the experimental supplement, Group C, none had died on the 20th day. In this latter group all showed extremely hunched

backs for many days and one had extreme paralysis and whirling syndrome on the 20th day and died on the 21st day. The filtrates from which the concentrates were prepared had been obtained by passing portions of digests containing about 6 micrograms of thiamine through 7- to 8-cm. (1.0 to 1.3 gram columns of Decalco. These conditions are near the "preferred" for cereals as given in the directions by the Research Corporation Committee (30), but apparently they were not the optimum for the adsorption of thiamine from the beef digests.

To obtain further evidence about the efficiency of adsorption in relation to various concentrations and various volumes of

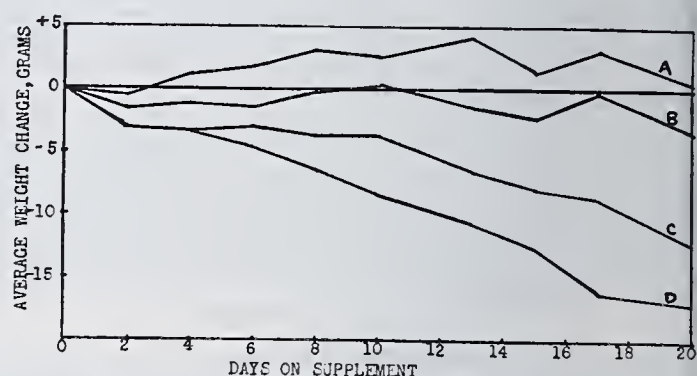


Figure 1. Average Weight Changes in Rats

In a 20-day supplementing period:

- A. 4 rats received daily 1.2 ml. of concentrate treated with  $\text{Na}_2\text{SO}_3$  to destroy thiamine, and 1  $\gamma$  of crystalline thiamine in 10% alcohol.  
 B. 6 rats received daily 1.2 ml. of concentrate with crystalline thiamine added before concentration in an amount equivalent to 1  $\gamma$  per 1.2 ml. of concentrate.  
 C. 5 rats received daily 1.2 ml. of concentrate being assayed for thiamine activity.  
 D. Started with 5 rats, received daily 1.2 ml. of concentrate treated with  $\text{Na}_2\text{SO}_3$ .

Table III. Effect of Hydrogen Peroxide in the Harris Thiochrome Reaction on Beef Digests (Sample 3)

Digest No.	7/28/42 Digests		8/10/42 Digests	
	No $\text{H}_2\text{O}_2$	5 drops 5% $\text{H}_2\text{O}_2$	No $\text{H}_2\text{O}_2$	1 ml. 30% $\text{H}_2\text{O}_2$
	$\gamma/g.$	$\gamma/g.$	$\gamma/g.$	$\gamma/g.$
1	1.72	1.44	1.45	1.49
2	1.53	1.51	1.46	1.47
3	1.52	1.50	..	..
4	1.70	1.65	..	..

Table IV. Hennessy Thiamine Values

(In relation to total amount of thiamine placed on a Decalco column. Obtained in several experiments on a raw beef round, sample 8.)

Beef Concentration $G/ml.$	Volume Adsorbed $ml.$	Thiamine Placed on Column <sup>a</sup> $\gamma$	Thiamine Determined $\gamma/g.$	Recovery Crystalline Thiamine %
0.0332	25	1.07	1.27	...
0.0373	25	1.20	1.29	102
0.0421	25	1.36	1.28	...
0.0412	40	2.12	1.29	...
0.0332	50	2.15	1.18	100
0.0682	25	2.20	1.22	96
0.0705	25	2.28	1.28	97
0.0373	50	2.41	1.26	88
Av. 1.27 $\pm$ 0.017 (omitting 5)				
0.0982	25	3.16	1.19	...
0.0373	75	3.60	1.25	91
0.0421	75	4.07	1.19	...
0.1333	25	4.29	1.19 <sup>c</sup>	...
0.1333	25	4.29	1.25 <sup>c</sup>	...
0.0682	50	4.39	1.11	...
0.0366	100	4.72	1.16	...
0.0373	100	4.81	1.16	...
0.0378	100	4.88	1.11	...
0.0393	100	5.06	1.19	...
0.0982	50	6.33	1.02	...
0.0982	50	6.33	1.05	...
0.1333	40	6.87	0.91 <sup>c</sup>	...
0.1333	40	6.87	1.12 <sup>c</sup>	...

<sup>a</sup> Computed by use of highest value, 1.29  $\gamma$  per gram, ever obtained this sample.<sup>b</sup> One or 2  $\gamma$  added to another column with same quantity of beef digest.<sup>c</sup> Made with 0.05 N HCl, all others with 0.1 N  $\text{H}_2\text{SO}_4$ .



concentration, the experiments presented in Tables IV and V were carried out. The two beef samples used in these experiments were higher in thiamine content than the rib used in the rat experiment about 35% in the case of sample 8 (Table IV), and about 6% in the case of "heel of round" (Table V). Hence, the proportion of interfering material to thiamine might have been appreciably lower in the former at least. Nevertheless, the results on this show that the trend of thiamine values is toward lower values as larger amounts, up to about 7 micrograms of thiamine, are placed on the 7-cm. (1.0 to 1.3 gram) Decalso column; that the gradation toward lower values is not absolutely consistent with increasing amounts adsorbed; but that when adsorption was in the range of 1 to 2 micrograms the average of seven values,  $1.27 \pm 0.017$  micrograms per gram (omitting one which was in less good agreement) is appreciably higher than most values obtained with adsorption of larger amounts. In the range of 3 to 5 micrograms per column there is some overlapping of values with the first group, but most of the ten determinations in this range were 6 to 9% lower than the above average and two were almost 13% lower. With 6 to 7 micrograms placed on a column the values were 12 to 28% lower.

In the experiment with the "heel of round" sample (Table V) possible variations due to sampling and extraction were eliminated, for all adsorptions were done from one large digest. Also, standard recovery determinations were carried to higher levels of adsorptions and Harris thiochrome determinations were included on at least one of every pair of adsorption filtrates to discover whether there was any inverse correlation of these with the decreasing Hennessy values on eluates. The downward trend in Hennessy values was even more rapid in this sample, being 10 to 15% at the level of 2.7 micrograms of beef thiamine per column, and 18 to 23% at 4.05-microgram level. The decrease was not, however, correspondingly greater above 4 micrograms, averaging around 20 to 25% but reaching an extreme of 31% in one determination with 8.1 micrograms per column. However, the recovery of an added 2 micrograms of synthetic thiamine per column was decidedly reduced when the beef thiamine was above 5 micrograms as compared to the recoveries at intermediate levels. The latter, in fact, appear to be misleading in their highness when based on beef values obtained at the same level of adsorption where apparently there was 10 to 20% loss of beef thiamine.

There is no doubt that the amounts of thiamine as measured by the Harris thiochrome reaction carried out in the adsorption filtrates show an upward trend as Hennessy values become lower, and though the correlation is not perfect, this finding makes it more conclusive that the major loss at higher levels is in the adsorption. Physical interference with photofluorometry at higher levels is ruled out by the fact that "internal standards" added to eluates are used for calculations of all Hennessy values, high and low, and such internal standard readings average about the same in aliquots of eluates from the larger adsorptions as in those from the smaller ones.

Obviously, then, in beef digests, interfering material is present in sufficient amount so that it is necessary to adsorb under 2.5 micrograms per column containing 1 to 1.3 grams of Decalso, to be assured of 90 to 100% adsorption. The extraction seems to

Table V. Hennessy Thiamine Values

(Obtained on one digest of raw beef<sup>a</sup> with accompanying Harris values on filtrates from adsorption at several levels. Beef concentration in digest: 0.0551 gram per ml.)

Decalso Column No. <sup>b</sup>	Volume of Digest Adsorbed, ml.	Thiamine Placed on Column From beef <sup>c</sup> , $\gamma$	Thiamine Added crystalline, $\gamma$	Hennessy Thiamine Values Found for Beef, $\gamma/g.$	Recovery of Added Thiamine <sup>d</sup> , %	Computed Loss in Hennessy Determination <sup>e</sup> , $\gamma/g.$	Harris Values on Adsorption Filtrates, $\gamma/g.$
1	25	1.35	..	0.98	..	0	..
2	25	1.35	..	0.94	..	0.04	0.14
3	25	1.35	2.0	..	95	..	..
4	25	1.35	2.0	..	92	0.14	0.16
5	25	1.35	..	0.93	..	..	..
6	25	1.35	..	0.87	..	0.10	0.13
7	25	1.35	2.0	..	92	0.19	0.18
8	25	1.35	2.0	..	98	..	..
9	50	2.70	..	0.81	..	0.18	0.32
10	50	2.70	..	0.82	..	..	..
11	50	2.70	2.0	..	99	0.17	0.26
12	50	2.70	2.0	..	104	..	..
13	75	4.05	..	0.72	..	0.26	0.23
14	75	4.05	..	0.76	..	..	..
15	75	4.05	2.0	..	98	0.23	0.26
16	75	4.05	2.0	..	98	..	..
17	100	5.40	..	0.70	..	0.27	0.35
18 <sup>f</sup>	100	5.40	..	0.73	..	..	..
19	100	5.40	2.0	..	47	0.47	0.56
20	100	5.40	2.0	..	73	0.34	0.35
21	150	8.10	..	0.66	..	0.32	0.38
22	150	8.10	..	0.76	..	0.22	0.40
23	150	8.10	2.0	..	59	0.42	0.40
24	150	8.10	2.0	..	39	0.37	0.51

<sup>a</sup> "Heel of round" cut.

<sup>b</sup> Lengths of Nos. 1-4, inclusive, were 13 to 14 cm. (2.2 to 2.4 grams of Decalso); all others 7 to 8 cm. (1.1 to 1.3 grams).

<sup>c</sup> Computed by use of highest Hennessy value, 0.98 $\gamma$  per gram.

<sup>d</sup> Based on average of two values on beef alone obtained at corresponding level of adsorption, except columns 19, 20, 23, and 24, where recovery was based on single determination with corresponding elution volume. See *f*.

<sup>e</sup> Based on highest Hennessy value and same plus added thiamine.

<sup>f</sup> Volumes of elutions with boiling hot KCl-HCl were 25 ml. in all except: 18, 40 ml.; 20, 40 ml.; 22, 50 ml.; 24, 50 ml.

Table VI. Comparison of Thiamine Values by Yeast Fermentation and Thiochrome Methods

Sample	Date	Harris Thiochrome, $\gamma/g.$	Hennessy Thiochrome, $\gamma/g.$	Schultz and Frey Yeast Fermentation, $\gamma/g.$
3, raw frozen	8/10/42	1.55	1.11	..
	8/31/42	..	..	1.22
4, raw fresh	8/25/42	0.82	0.49	..
4, raw frozen	8/31/42	..	..	0.60
4, cooked fresh	8/25/42	0.66	0.45	..
4, cooked frozen	8/31/42	..	..	0.34
7, raw frozen	2/12/43	1.16	0.91	0.91
8, raw frozen	2/12/43	1.34	1.19	1.22

be equally good in more concentrated digests—that is, in those containing 0.07 to 0.09 gram of beef per milliliter—as in those which are from one half to one third as concentrated. Therefore the more practical technique would seem to be adsorption of small volumes, 20 to 25 ml., of digests which contain from 1 to 2.5 micrograms in the total volume adsorbed.

There is no obvious reason for the relatively lower differences between Harris and Hennessy values found in the last samples of rib and round as compared to those in the earlier ones. It would seem that there must be variation in the occurrence of that factor which is responsible for the discrepancies between the fluorescence readings obtained after direct ferricyanide oxidation of digests and that following adsorption. Since there is no reason to believe that adsorptions were any more complete in the Hennessy assays of samples 7 and 8 than in earlier ones, such an explanation cannot be taken as the reason for the two values coming closer together. Besides, in most adsorptions of the first five samples the amounts of thiamine placed on a column were under 2.7 micrograms, reaching as high as 4.0 and 4.5 in the case of only a couple of the adsorptions of the second experiment on sample 3. Hence, throughout this series, at worst, adsorptions could be considered to be 10 to 20% too low and thus not so poor



as to explain large proportions of the discrepancies between the two thiochrome values.

While making the attempts just described to obtain proof for the validity of either the direct oxidation or the adsorption technique for the thiochrome method, the authors thought that a comparison of values on the same samples by the yeast fermentation method might lend support to one of the thiochrome techniques. They were fortunate to find Dr. Schultz in Dr. Frey's laboratory willing to make such determinations. His results are tabulated in Table VI with the corresponding Hennessy and Harris values on frozen samples taken from Table I. As may be seen, his are, in several instances, somewhat higher than the Hennessy values but they are decidedly closer to the Hennessy than to the Harris values in those cases where there is a large difference between the latter two.

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## Constant-Level Feeder for Continuous Evaporation in the Determination of Total Solids

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THE apparatus shown in the figure was developed for use in the determination of total dissolved solids in water, and built to the authors' specifications by the Scientific Glass Apparatus Company. An apparatus was desired which could be assembled and started quickly; the apparatus herein described has proved successful in a number of actual tests.

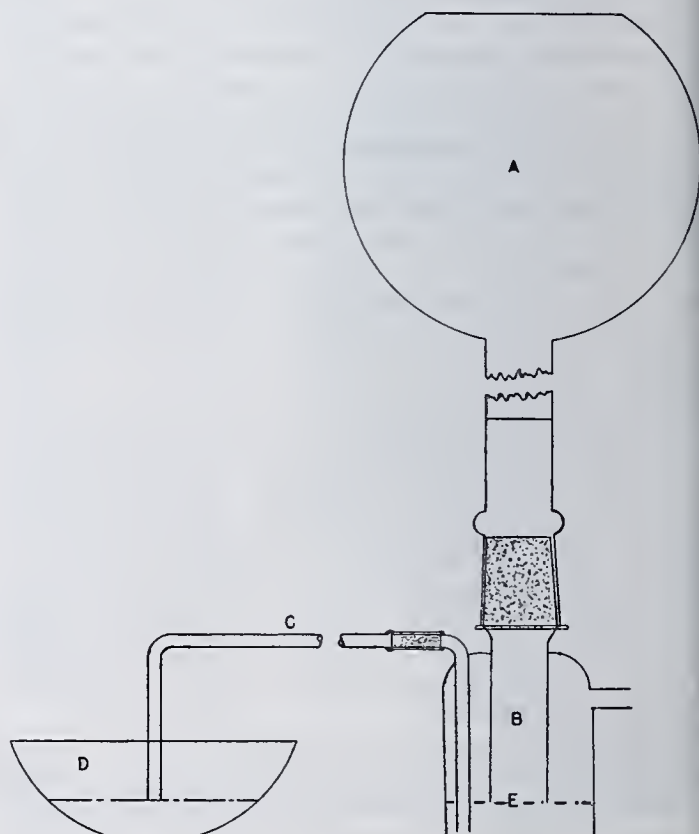
The evaporator maintains a constant level of liquid in the evaporating dish, *D*, for continuous evaporation. The volumetric flask, *A*, is filled to the mark with the solution to be evaporated, *B* is put in place, and the unit is then turned over as illustrated. The liquid rises in *B* to height *E* and then ceases to flow. *C* and *D*, the evaporating dish, are added. The solution is siphoned over through *C* and stops flowing when the level in *D* reaches a height equal to *E*. The siphon can be started by blowing in the vent of *B*. As evaporation proceeds, more liquid flows over, maintaining the original level in the dish. Any slight amount of liquid finally left in *C* or *B* can be emptied manually.

The evaporation can be carried out on a water bath, steam bath, or electrical hot plate. Under certain conditions, glass in *C*, in contact with the liquid in the evaporating dish, will be undesirable when extremely pure liquids are evaporated. In such instances resistant glass, Vycor, fused quartz, or metal tubing (suitable for the liquid) can replace conventional Pyrex tubing.

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# Rapid Photometric Determination of Total Nitrogen, Phosphorus, and Potassium in Plant Material

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id photometric methods for the determination of total nitro-  
phosphorus, and potassium in plant material are presented.  
plant material is rapidly ashed by means of sulfuric acid and  
hydrogen peroxide. Tests for the three elements are run on separate  
quots of the ash extract by means of a photoelectric colorimeter.  
mparison with A.O.A.C. methods shows fairly good agreement.  
hough not so accurate as the A.O.A.C. methods, these rapid  
hods provide for a very rapid determination of nitrogen, phos-  
phorus, and potassium with sufficient accuracy for many routine  
poses.

HE ash analysis of plant tissue yields much information as  
to the nutritional history of plants, affords an estimate as to  
rate and total amount of nutrients removed from the soil, and  
ps the agronomist to meet this nutrient removal. In recent  
rs, the accurate ash analysis of specified plant tissue has been  
d to ascertain the nutritional status of the plant (6, 7). A  
liography covering recent investigations in ash analysis has been  
pared (4).

The results of many more ash analyses of plants would be of  
at importance to agriculture. However, the use of this type of  
alysis of plant tissue has been somewhat limited by the length  
ime required for such determinations.

The ashing of plant material can be speeded up considerably by  
e use of sulfuric acid and hydrogen peroxide. This method has  
en used for the determination of phosphorus (5) and nitrogen,  
d has been suggested for other nutrients (2).

Rapid tests for soluble plant nutrients have been used to prac-  
l advantage (8), but are of limited value. They give the  
ounts of soluble nutrients only; they do not yield a complete  
tory of plant nutrition, nor give the amounts or rates of nu-  
ent removal. The need for knowing the total constituents in  
nt material, and for obtaining this information for a large  
mber of samples in a short time, has led to the development of  
e rapid tests described in this paper.

These methods are based on a rapid system of wet-ashing (2)  
d rapid photometric methods. They have been used with suc-  
ss on peas, beets, spinach, crimson clover, and rye. Their  
ief advantage lies in the speed of determination (it is possible  
an analyst to determine the total amounts of nitrogen, phos-  
phorus, and potassium in 24 ground samples in 1 day). Added  
vantages are in the economy of reagents and labor. A large  
mber of reagents prepared for the analysis of soluble nutri-  
ts in soils and in plant extracts (8) may also be used in these  
ethods. A laboratory equipped to determine soluble nutrients  
n also determine ash constituents of plants without great addi-  
onal expense for equipment or reagents.

## APPARATUS

In addition to the apparatus suggested for the determination of  
uble nutrients in soil and plant extracts (8), the following are  
eded: plant mill, hot plate, and Erlenmeyer flasks, 50 ml.  
arked at 50 ml.

## REAGENTS AND SOLUTIONS

All reagents are of C.P. grade. Wherever possible, Baker's  
alyzed reagents are used.

ASHING. Sulfuric acid, concentrated, nitrogen-free. Hydro-  
n peroxide, 30%.

PREPARATION OF ASHED MATERIAL. Morgan's Universal ex-  
tracting solution (3), 0.5 N acetic acid buffered at pH 4.8 with so-  
um acetate, hereafter referred to as extracting solution.

DETERMINATION OF NITROGEN. Graves' reagent (9), 80 grams  
of sodium chloride dissolved in 130 ml. of water, to which are added  
100 ml. of a cold saturated solution of mercuric chloride (7%)  
with shaking. The salt is almost dissolved and 70 ml. of a satu-  
rated solution of lithium carbonate (1%) are added in small quan-  
tities and with continued shaking. Five grams of talc are added  
to the solution which is filtered, stored in a brown bottle, and kept  
stoppered. The solution will keep for several weeks. Shake be-  
fore using.

Gum arabic, 0.25% solution. Sodium hydroxide, 15%.

Standard nitrogen. Ammonium chloride in extracting solution  
to supply 10 p.p.m. of nitrogen.

Detailed directions for preparing the solutions listed below  
have been given (8).

DETERMINATION OF PHOSPHORUS. Ammonium molybdate,  
2.5% in 6 N sulfuric acid. Aminonaphthol sulfonic acid.

Standard phosphorus. Monosodium phosphate monohydrate,  
dissolved in extracting solution to give 20 p.p.m.

DETERMINATION OF POTASSIUM. Sodium cobaltinitrite.  
Isopropyl alcohol (no formaldehyde). Gum arabic solution (as  
for nitrogen).

Standard potassium. Potassium chloride in extracting solu-  
tion to supply 50 p.p.m.

## METHODS

ASHING. A 0.200-gram sample of finely ground, well-mixed,  
plant material is placed in a 50-ml. Erlenmeyer flask, marked at  
50 ml., and 3 ml. of concentrated sulfuric acid are added. The  
flask is rotated to mix the plant material with the acid. A small,  
short-stem funnel is placed in the neck, and the flask is heated on  
a hot plate for about 5 minutes after fuming starts. The tempera-  
ture of the hot plate is adjusted so that fumes are given off, but  
are not driven from the flask. The flask is removed from the  
hot plate and allowed to cool for a few minutes, and 1 ml. of 30%  
hydrogen peroxide is slowly added, dropwise, to the sides of the  
funnel and flask. Slow addition in this manner avoids spattering  
and washes down charred material adhering to flask or funnel.  
The flask is reheated for about 2 minutes. If the material is still  
dark, the flask is cooled, rotated, and 5 additional drops of hy-  
drogen peroxide are added as before. The flask is again heated.

This process of adding 5 drops and reheating is repeated until  
the solution is colorless, then the solution is heated slowly for 5  
minutes to expel excess hydrogen peroxide. Removing the funnel  
prior to the last heating will aid in the expulsion of the hydrogen  
peroxide, but care in heating must be exercised to avoid loss of the  
liquid. The flasks are cooled, extracting solution is added to the  
mark, and the contents are filtered on a Whatman No. 2 filter  
paper to remove silica. Aliquots of the filtrate (referred to as ash  
extract) are used for the determination of nitrogen, phosphorus,  
and potassium. (Where many samples are being run it is con-  
venient to allow the silica to settle out in the flask. Aliquots are  
taken of the supernatant liquid without disturbing the precipi-  
tate on the bottom.)

Table I. Determination of Nitrogen, Phosphorus, and Potassium for  
Standard Curves and in Plant Material

Nutrient Deter- mined	Material	Useful Range P.p.m.	Volume of Aliquots ML.	Diluted to ML.	Filter Used	Null Adjust- ment with Blank to
Nitrogen	Standard solution	0 to 8	0 to 16	20	425 (blue)	100
	Ash extract	20 to 160	1			
Phos- phorus	Standard solution	0 to 12.5	0 to 12.5	20	425 (blue)	0 (log scale)
	Ash extract	1 to 50	5			
Potassium	Standard solution	0 to 20	0 to 8	10	650 (red)	100
	Ash extract	50 to 200	1			



**CALIBRATION OF STANDARD CURVES.** Standard curves for nitrogen, phosphorus, and potassium are prepared by adding a series of aliquots of standard solutions and proper amount of the blank to photometer tubes, diluting to appropriate levels (Table I), and treating as for the determination of the elements in the ash extract. Photometer readings are taken with a Fisher electrophotometer using the appropriate filters (Table I). Concentration deflection curves are drawn from the resultant readings.

A suitable blank is prepared by ashing 0.200 gram of pure sucrose in the same manner as the plant material, and the ash is diluted to 50 ml. with extracting solution. Aliquots of this blank are added to each of the standards as follows: 1.0 ml. for the nitrogen, 5 ml. for the phosphorus, and 1.0 ml. for the potassium determination.

**DETERMINATION OF NUTRIENTS. Nitrogen.** To a series of photometer tubes 1.0-ml. aliquots of the ash extracts are added, the contents are diluted to 20 ml. with extracting solution, and 0.2 ml. of gum arabic solution is added to each. The contents are mixed by means of a flat-bottomed rod and 0.5 ml. of Graves' reagent is added. The contents are again mixed by means of the flat-bottomed rod and 5 ml. of 15% sodium hydroxide are added, mixing again after the addition of the hydroxide. Photometer readings are taken in exactly 15 minutes. A 425 blue filter is used and the null is adjusted to 100% transmission with the blank.

**Phosphorus.** Aliquots of the ash extracts (5 ml.) are pipetted into a series of photometer tubes, the contents are diluted to 20 ml. with extracting solution, and 4 ml. of ammonium molybdate solution and 2 ml. of aminonaphthol sulfonic acid solution are added. The contents are stirred and allowed to stand for 15 minutes. Photometer readings are taken, using a 425 blue filter and adjusting the blank to 0 (log scale).

**Potassium.** Aliquots of the ash extract (1 ml.) are pipetted into a series of photometer tubes, the contents are diluted to 10 ml. with extracting solution, and to this is added 0.5 ml. of gum arabic solution. The tubes are rotated, avoiding any loss of the liquid, then 2 ml. of sodium cobaltinitrite solution are pipetted directly into the contents of each tube. The tubes are rotated to mix the contents, allowed to stand for 5 minutes, and 10 ml. of isopropyl alcohol are run directly into the solution. (The isopropyl alcohol should always be added from a uniform dispensing unit, such as an automatic pipet, and from a uniform height.) The tube is stoppered, inverted 3 times, and allowed to stand for 15 minutes. The stopper is then removed and photometer readings are taken, using the 650 red filter and adjusting the blank to 100% transmission.

**CALCULATIONS.** Using the photometer readings obtained in the tests, the concentrations in p.p.m. can be read directly from the standard curves or from charts prepared from such curves.

#### DISCUSSION OF METHODS

**SELECTION AND PREPARATION OF SAMPLES.** Samples dried at 40.56 °C. (105 °F.) should be finely ground (100% to pass a 0.5-mm. sieve) and thoroughly mixed. As in all cases where small aliquots are taken from large samples, it is important that the sample should be finely ground and uniformly mixed.

Selection of the portion of the plant to be used for testing depends upon the purpose of the investigation and the type of plant tested. Investigations as to the status of nutrition have employed the use of leaf material (6, 7). The entire plant should be analyzed for determining the amounts and rates of removal of nutrients by crops.

**CALIBRATION OF STANDARD CURVES.** The reactions for the tests are influenced considerably by pH changes. Use of the buffered extracting solution helps to maintain a more uniform pH value. However, since the addition of the ash extract to extracting solution does cause a change in the pH, it is best compensated for by adding a similar aliquot of the blank extract in the calibration of the standard curves—for example, a 5-ml. aliquot of the ash extract is used in the phosphorus determination. A similar aliquot of the blank extract should be added to each aliquot of the standard and the contents diluted to 20 ml.

**TESTS.** Aliquots of the ash extract of the blank should be of the same volume and treated in the same manner as the aliquots of the plant material.

A blank should be run with all determination. The ashing by means of sulfuric acid and hydrogen peroxide effects solution of the elements in question, with the nitrogen present in ammonium form. Silica precipitates and can be removed by filtration or ali-

quots of the ash extract may be removed from the supernatant liquid without disturbing the precipitate.

In the nitrogen determination, care should be taken to heat the sample initially for about 5 minutes after fumes appear to prevent loss of nitrate nitrogen (2) and aid in decomposition of the complex organic nitrogen compounds. Baking should be avoided since it will drive off nitrogen. A large excess of hydrogen peroxide in the ashing will cause an appreciable loss of nitrogen.

The precipitate formed by addition of the Graves' reagent and sodium hydroxide is greatly influenced by the time of standing and pH. Repeatable results can be obtained by taking readings exactly 15 minutes after the addition of the sodium hydroxide. Changes in the pH value due to addition of the sample are compensated for by similar additions of the blank to the standards in the preparation of the standard curves.

**Table II. Determination of Total Nitrogen, Phosphorus, and Potassium in Plant Material**

[Comparison of rapid photometric methods with A.O.A.C. (1) method]			
Nutrient	Material	Rapid Methods % <sup>a</sup>	A.O.A.C. Method % <sup>a</sup>
Nitrogen	Wheat straw	0.4	0.308
	Alfalfa	2.4	2.82
	Cottonseed meal	7.0	7.25
	Soybean meal	7.5	7.69
Phosphorus	Starter mash 1	1.01	1.08
	Starter mash 2	0.66	0.70
	Starter mash 3	0.82	0.77
	Starter mash 4	0.68	0.69
	Starter mash 5	0.81	0.77
	Starter mash 6	1.00	1.04
Potassium <sup>b</sup>	Starter mash 1	1.1	0.95
	Starter mash 2	0.8	0.75
	Starter mash 3	0.7	0.69
	Starter mash 4	0.7	0.75
	Starter mash 5	0.7	0.86
	Starter mash 6	0.9	0.82

<sup>a</sup> Per cent of plant material on a dry basis.

<sup>b</sup> Corrections made for ammonium ion present.

The gum arabic gives a more uniform dispersion, and also enables the determination of larger amounts of nitrogen.

The readings are most accurate for nitrogen in amounts from 0.5 to 8 p.p.m. With a 1.0-ml. aliquot, this represents from 0.5 to 4% of total nitrogen in the dry tissue; most plant samples will fall within this range. For material containing less than 0.5% total nitrogen, a standard amount of nitrogen can be added to the ash extract aliquot and subtracted from the results.

The phosphorus determination is only slightly affected by pH changes. Such changes are compensated for by adding a similar quantity of blank to the standard in preparation of standard curves.

The phosphorus test allows for the determinations of phosphorus in amounts from 0.25 to 12.5 p.p.m. This represents 0.025 to 1.25% total phosphorus in the dry plant material, if a 5-ml. aliquot of the ash extract is used. Most plant material will contain phosphorus in amounts between these figures.

The potassium determination is influenced by changes in pH value, by the rate of mixing the alcohol with the cobaltinitrite by temperature changes, and ammonia present.

The influence of pH is nullified by taking the same amount of aliquots in all cases and by adding similar amounts of the blank to the standards.

The colloidal precipitate formed by the addition of the alcohol is greatly influenced by the speed with which the alcohol is added and subsequent shaking thereof. Gum arabic aids in the formation of a more uniform precipitate. However, constantly obtaining uniform precipitates, the alcohol should be delivered from the same apparatus at a constant height above the contents in the photometer tube. By adding the alcohol from a 3-way, 10-ml. automatic pipet directly into the contents and immediately stoppering and inverting 3 times, fairly uniform precipitates were obtained.



ined. Previously (8) alcohol was added slowly down the sides of the tube and rotated. The present method is more accurate, since there is less dependence on the individual operator.

If large changes in temperature take place in the laboratory, separate standard curves should be drawn for every 5° C. This seems simpler than cooling to a standard temperature.

In previous methods (8), formaldehyde was used to avoid interference of ammonia. Formaldehyde lessens interference from ammonia but also reduces the sensitivity for the determination of potassium in lower concentrations. In the present method, there is no provision for avoiding the interference of ammonium ion in the test but a correction for the ammonium ion is made. Curves can be drawn showing the influence of adding standard amount of ammonium ions to definite amounts of potassium and deductions made according to such graphs. For practical purposes an accurate correction can be made by use of the following equation:  $X = 0.16(A)(B)$  where  $X$  = % potassium to be deducted,  $A$  = % total nitrogen, and  $B$  = % potassium (uncorrected), all on a dry weight basis. This correction is based on a 1-ml. sample of the ash extract.

The potassium test is satisfactory for potassium in amounts of 5 to 20 p.p.m. If a 1-ml. sample is used this represents 1.25 to 20% total potassium in plant material. For amounts less than 25% potassium, a standard amount of potassium is added to the aliquot and later deducted from the results.

**ACCURACY.** The nitrogen test can be repeated with an accuracy of 0.2 p.p.m., providing the final ammonium nitrogen content is between 0.5 and 4 p.p.m. This represents 0.2% of total nitrogen on a dry weight basis for amounts between 0.5 and 4% of nitrogen. Comparison of the rapid method (Table II) with the A.O.A.C. method shows fairly good agreement.

Phosphorus determinations can be repeated within 0.2 p.p.m.

Based on a 5-ml. aliquot, this represents 0.02% total phosphorus in the plant material. Phosphorus determinations of starter mash agreed very closely with A.O.A.C. values (Table II).

The potassium test is perhaps the least accurate of the three, primarily because it is influenced by so many factors. However, if precipitated under uniform conditions and effect of temperature and ammonium ion are taken into consideration, fairly accurate results can be obtained. The test can be repeated within 1 p.p.m. in a range of 5 to 20 p.p.m. Based on a 1-ml. sample, this represents 0.25% potassium in a total potassium content of a plant material from 1.25 to 5.0%. Agreement with the A.O.A.C. method (Table II) was fair.

#### ACKNOWLEDGMENT

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## Photoelectric Photometry

### An Analysis of Errors at High and at Low Absorption

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Mathematical and experimental proofs show that errors in setting the zero point and  $I_0$  on the galvanometer, and in reading the galvanometer deflection for transmitted light, produce high relative errors when a photoelectric photometer is used with solutions of high or of low absorption. For maximum accuracy, conditions should be so chosen that readings of transmitted illumination fall on the central portion of the scale.

**N**UMEROUS photoelectric devices are in use in chemistry laboratories throughout the country. Most of them ("photoelectric colorimeters") are used for measuring the relative amount of light transmitted by a given depth of colored liquid in order to determine the concentration of solute which absorbs light of a certain wave length. The light used in measurement is restricted to more or less narrow bands by means of filters, prisms, or gratings. Measurement is effected by reading the deflection of a galvanometer caused by a photoelectric cell, or by use of a potentiometer to balance the circuits of two photoelectric cells.

Several articles dealing with errors involved in the use of these instruments have appeared. Two such articles have dealt with errors produced by wide transmission bands and other sources of stray light (1, 4). The most satisfactory means of meeting this deficiency and controlling the errors arising from it has been to construct empirical analytical curves relating galvanometer

readings or logarithms thereof to respective concentrations of solute. Elimination of errors of this type, the result of instrument limitations, remains a problem for instrument designers and manufacturers.

There remain, however, several sources of error which can be minimized by proper use of available instruments, provided the user is aware of the existence of these errors and of the precautions necessary to make them as small as possible. Most chemists know in a general way that maximum accuracy cannot be achieved when galvanometer readings are either very high or very low. The magnitude of the errors which may occur at each end of the scale makes an analysis of them desirable.

When approximately monochromatic light of a wave length in the region of light absorption of the solution is used, the Beer-Lambert law applies closely to most colored solutions. Let us assume conditions such that the law is valid, and further assume that galvanometer deflection is proportional to the intensity of light striking the photocell. Then

$$y = \frac{I}{I_0} = e^{-kc} \quad (1)$$

where  $y$  is the ratio of the intensity of transmitted light,  $I$ , to the intensity of incident light,  $I_0$ ,  $e$  is the Napierian base,  $c$  is the concentration of light-absorbing molecules, and  $k$  is a positive constant determined by the nature of the solute molecule, the wave



length of light used, and the effective thickness of the liquid layer. Equation 1 is plotted in Figure 1.

#### ERROR IN THE GALVANOMETER READING FOR $I$

Suppose a small error,  $i$ , or  $\Delta y$ , either positive or negative, is made in the galvanometer reading. This error will cause an erroneous reading,  $I'$ , to be obtained for the transmitted light, such that

$$\frac{I'}{I_0} = e^{-k(c + \Delta c)} \quad (2)$$

For a given value of  $i$ ,  $\Delta c$  (the absolute error in  $c$ ) will be least for low concentrations of solute, since the curve is steepest here. However, in this region the relative value of the error,  $\frac{\Delta c}{c}$ , is large because of the smallness of  $c$ . For high values of  $c$ , the curve flattens out, and for a given value of  $i$ ,  $\Delta c$  becomes large, so that  $\frac{\Delta c}{c}$  again becomes large. Since minimum relative errors (percentage errors) are usually desired in analytical work, values of  $c$  which will produce the minimum value of  $\frac{\Delta c}{c}$  should be sought.

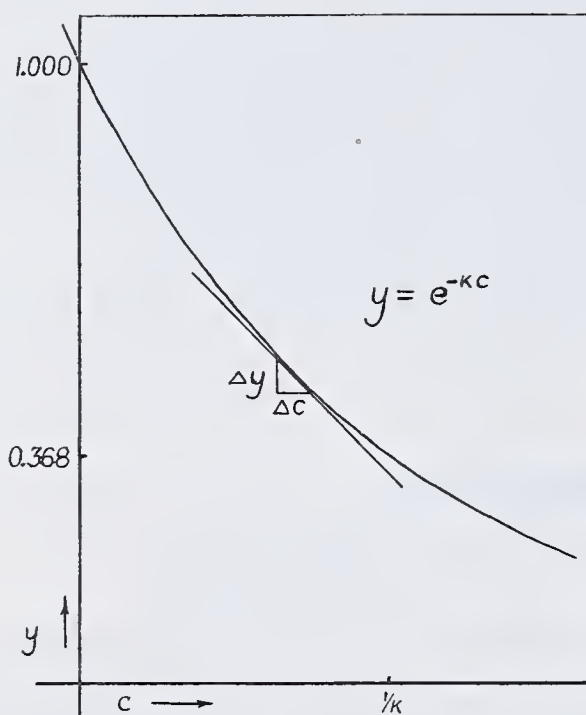


Figure 1. Relationship between Fraction of Incident Light Transmitted by the Solution (Ordinates) and the Concentration of Light-Absorbing Molecules (Abscissas)

The relationship of the erroneous reading,  $I'$ , to the true value,  $I$ , is as follows:

$$I' = I + i \quad (3)$$

whence

$$\frac{I'}{I_0} = \frac{I + i}{I_0} \quad (4)$$

Now

$$i = \Delta y = \frac{I'}{I_0} - \frac{I}{I_0} \quad (5)$$

and substituting from 4,

$$\begin{aligned} \Delta y &= \frac{I + i}{I_0} - \frac{I}{I_0} \\ &= \frac{i}{I_0} \end{aligned} \quad (6)$$

From Figure 1 it is apparent that slope  $m$ , of the curve,  $y = e^{-kc}$ , is equal approximately to  $\frac{\Delta y}{\Delta c}$ . Then

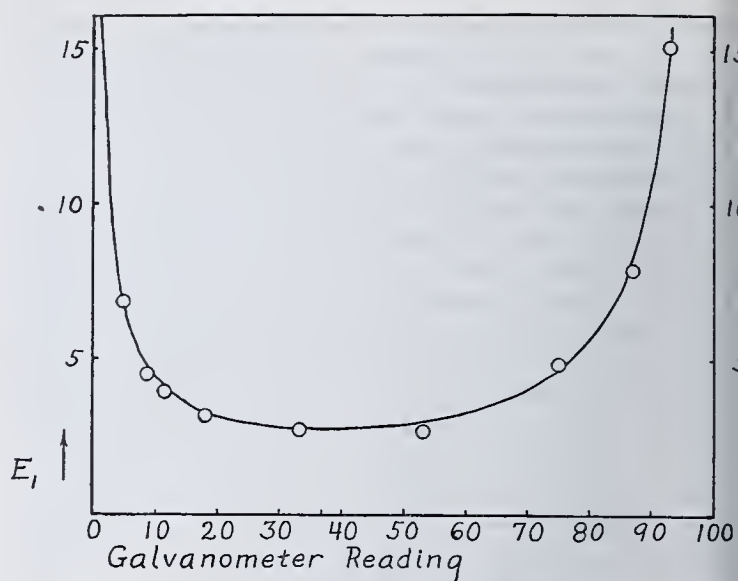


Figure 2. Relative Error in Results Obtained When a Given Error Is Made in the Reading for Transmitted Light

$$\Delta c = \frac{\Delta y}{m} \text{ (approximately)} \quad (7)$$

where

$$m = f'(c) = -ke^{-kc}$$

Designate the relative or "percentage" error in the concentration of the solute by  $E$ . Then from 7 and 6,

$$\begin{aligned} E &= \frac{\Delta c}{c} = \frac{\Delta y}{cm} \\ &= \frac{i}{I_0} \left( \frac{1}{-kce^{-kc}} \right) \end{aligned} \quad (8)$$

Since  $\log_e y = -kc$ ,

$$E = \frac{i}{I_0} \left( \frac{1}{y \log_e y} \right)$$

The fraction  $\frac{i}{I_0}$  is the ratio of the galvanometer error to the scale length.

The function  $E_1$ , defined by the relationship

$$\begin{aligned} E_1 &= -E \left( \frac{I_0}{i} \right) \\ &= \frac{-1}{y \log_e y} \end{aligned} \quad (9)$$

is plotted as the smooth curve in Figure 2, where abscissas are indicated as galvanometer readings (100  $y$ ). In this figure,  $I_0 = 100$ , and if  $i = 1$ ,  $E_1$  becomes the percentage error in the determination of  $c$ .

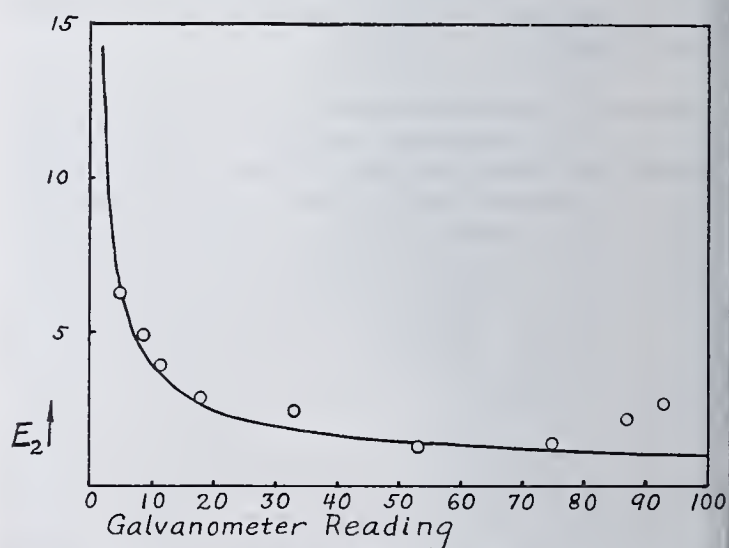


Figure 3. Relative Error in Results Obtained When a Given Error Is Made in Setting the Zero Point on the Galvanometer



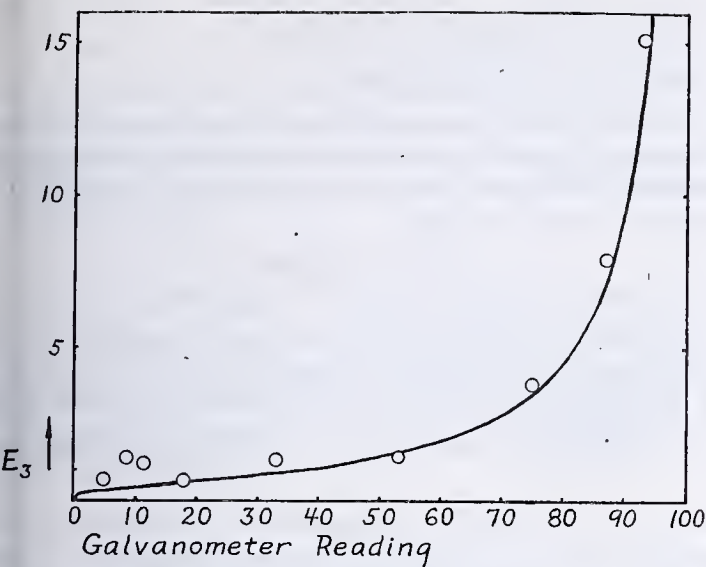


Figure 4. Relative Error in Results Obtained When a Given Error Is Made in Setting  $I_0$  on the Galvanometer

The function  $E_1$  has a minimum found by setting the derivative equal to zero and solving for  $c$ :

$$\begin{aligned} \frac{dE_1}{dc} &= \frac{d(y \log_e y)^{-1}}{dc} = \frac{d(k^{-1}c^{-1}e^{kc})}{dc} \\ &= \frac{1}{c} \left( 1 - \frac{1}{kc} \right) (e^{kc}) = 0 \end{aligned}$$

There is a real finite root at  $c = 1/k$ , or  $y = 1/e = 0.368$ . Thus, the minimum relative error in the determination of the concentration of solute, resulting from a random error in reading the galvanometer, is achieved when the reading is 36.8 ( $I_0 = 100$ ). Attention was called to this fact by Twyman and Lothian (5). Fogness, Zscheile, and Sidwell (1) published the curve in Figure 2 in a modified form, as did Ringbom (2). Schleicher (3) used a modification of Equation 9' in the calculation of average error over the useful portion of the error curve.]

If the base 10 is used instead of  $e$ , Equation 8 becomes

$$E = \frac{i}{I_0} \left( \frac{1}{-kc \cdot 10^{-0.4343kc}} \right) \quad (8')$$

Equation 9 becomes

$$E_1 = \frac{-0.4343}{y \log_{10} y} \quad (9')$$

The latter curve has a minimum at  $c = 1/k$ , or  $y = 10^{-0.4343} = 0.368$ .

It is interesting to note how steeply the error curve rises at high and at low galvanometer readings. Experimental verification of the importance of this curve is shown below.

#### ERROR IN SETTING ZERO POINT OF GALVANOMETER

When sensitive galvanometers are employed, the general procedure is to set the galvanometer at zero when no light is reaching the photocell or tube. With solvent or blank in the cuvette or absorption cell, the circuit is next adjusted so that the galvanometer reads 100 (incident illumination,  $I_0$ ). Following these settings, the light-absorbing solution replaces solvent or blank, and a galvanometer reading is made (transmitted illumination,  $I$ ). Above we have considered errors involved in this final reading. Errors in the first two settings are of the same magnitude as the latter, and are furthermore subject to possible increase during use of the instrument, owing to drift of the galvanometer and to fatigue and hysteresis of the photocell.

In considering errors arising from setting the zero point of the galvanometer, assumptions and notations are as above, except that  $i$  is now used to represent the error, positive or negative, in the zero point of the galvanometer. The relationship of the erroneous value,  $I'$ , to the true value,  $I$ , is as follows:

$$\frac{I' - i}{I_0 - i} = \frac{I}{I_0} \quad (10)$$

$$\frac{I'}{I_0} = \frac{I}{I_0} + \frac{i}{I_0} \left( 1 - \frac{I}{I_0} \right) \quad (11)$$

From 5,

$$\begin{aligned} \Delta y &= \frac{i}{I_0} \left( 1 - \frac{I}{I_0} \right) \\ E &= \frac{\Delta y}{mc} = \frac{i}{I_0} \left( \frac{1 - y}{y \log_e y} \right) \end{aligned} \quad (12)$$

The function  $E_2$ , defined by the relationship

$$E_2 = \left( \frac{y - 1}{y \log_e y} \right) \quad (13)$$

is plotted as the smooth curve in Figure 3.

#### ERROR IN THE GALVANOMETER SETTING FOR $I_0$

Notations are used as above, except that  $i$  now represents the error, positive or negative, in the setting of  $I_0$ , or resulting from drift from this setting. Now

$$\frac{I'}{I_0 + i} = \frac{I}{I_0}$$

whence

$$\frac{I'}{I_0} = \left( 1 + \frac{i}{I_0} \right) \frac{I}{I_0}$$

From 5,

$$\begin{aligned} \Delta y &= \frac{i}{I_0} \left( \frac{I}{I_0} \right) \\ E &= \frac{\Delta y}{mc} = \frac{i}{I_0} \left( \frac{1}{\log_e y} \right) \end{aligned} \quad (14)$$

The function  $E_3$ , defined by the relationship

$$E_3 = \frac{-1}{\log_e y} \quad (15)$$

is plotted as the smooth curve in Figure 4.

An interesting relationship now becomes apparent. Ordinates of the curve in Figure 2 can be obtained by adding corresponding ordinates of those in Figures 3 and 4. In other words,

$$E_1 = E_2 + E_3 \text{ (see Figure 5)}$$

Reflection shows that this relationship simply means that if an error in the same direction is made at both ends of the scale, the additive effect is to shift the whole scale, and to produce the same error in every reading of  $I$ .

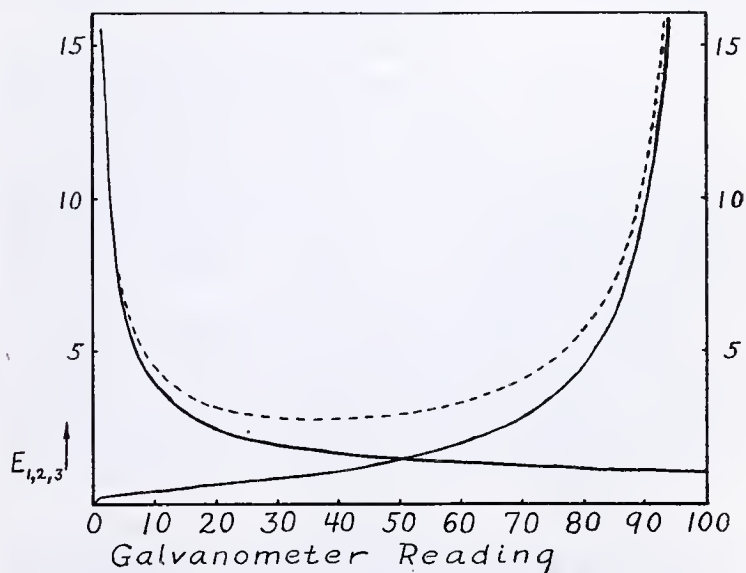


Figure 5. Relationship between Curves Shown in Figures 2, 3, and 4



These three curves of relative error emphasize the desirability of employing such conditions that galvanometer readings fall on the central portion of the scale. Only thus will be minimized the effect of three sources of error—namely, in settings of the galvanometer for zero and for incident illumination, and in readings of the values for transmitted illumination.

#### EXPERIMENTAL

Solutions of bromosulfalein were made up in 0.01 *N* sodium hydroxide, and light transmissions were determined with an Evelyn-type colorimeter, a light filter with maximum transmission at about 576  $m\mu$  being used. Readings were again determined with  $I_0$  set at 101 (+1% error in  $I_0$ ). The errors produced by this change were calculated on a percentage basis, under the assumption that the Beer-Lambert law was applicable. These errors are plotted as points in Figure 4.

Next the zero point of the galvanometer was set at 1 on the

scale, with  $I_0$  at 100. Readings were repeated. The percentage errors are plotted as points in Figure 3.

Finally the zero point was set at 1, and  $I_0$  was set at 101. Percentage errors are plotted in Figure 2.

It will be noted that random errors affect the conformation of the plotted points to the theoretical error curves, especially at the ends of the scale, where such errors have more weight. Otherwise agreement is good.

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## Extraction of Oil and Vitamin A in Shark Liver Analysis The Xylene-Centrifuge Method

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Xylene as a low-density solvent for the simultaneous centrifugal extraction and determination of oil and vitamin A is advocated for mass routine analysis of shark livers. The solvent need not be removed prior to the colorimetric determination of the vitamin by the Rosenthal-Weltner method. Moreover, the precision of the method for both oil and vitamin A is from 1 to 2% for most samples encountered during a large routine run.

IT OCCURRED to the author that a solvent having a relatively low rate of evaporation would afford more ease and less error in handling aliquots of the extracts than would solvents hitherto employed. This is especially important on warm days. A low density would permit use of centrifugation and would result in a clear supernatant extract. The solvent selected should exercise no deleterious effect on the vitamin A-antimony trichloride color, thereby allowing addition of the solvent containing the oil directly to the chloroform solution of antimony trichloride. Finally, the transmittancy of the solvent to light in the spectral range used for the assay should be high.

Purified xylene possesses these properties and in addition is less toxic and permits a lower unit cost per analysis than is true of other solvents used in vitamin A analysis. Its mutual solubility with water is low, thus obviating the use of dehydrating agents.

#### EXPERIMENTAL

"Xylene (xylol) pure" as obtained from supply houses is found to contain a considerable quantity of interfering materials. However, a good grade of solvent with excellent keeping qualities can be obtained by the following treatment. The crude xylene is first washed with concentrated sulfuric acid until no further charring occurs and then with water. The solvent is then treated with 10 to 20% sodium bisulfite, washed with water, dried over anhydrous sodium sulfate, and distilled in an all-glass still. The fraction boiling at 134° to 142° C., with a yield of 75 to 80%, is collected for the vitamin A and oil extraction.

The Rosenthal-Weltner reaction (1) was measured in a Klett-Summerson photoelectric colorimeter using a green (No. 54) filter. The direct absorption at 328  $m\mu$  was determined with a Beckman quartz prism spectrophotometer using isopropanol as the solvent. The transmission curves of the Rosenthal-Weltner reaction were obtained on a Coleman 10-S spectrophotometer (5  $m\mu$  slit width).

In order to test the effect of xylene on the transmission curve of the Rosenthal-Weltner color a natural vitamin A ester concentrate (200,000 I.U. per gram) was diluted 1 to 100 in chloroform as well as in xylene. To 2 ml. of each solution contained in Coleman tubes (1.6 mm. in inside diameter) were added 2 ml. of 0.5% guaiacol-chloroform solution and 6 ml. of 30% antimony

trichloride-chloroform reagent. The corked tubes were placed in a 62° C. water bath for 0.75 minute, then cooled to room temperature under the tap. Percentage transmissions, referred to blank consisting of the reagents and the appropriate solvent instead of the oil solution, were taken at intervals of 10  $m\mu$  in the range 500 to 575  $m\mu$ . The results are shown in Table I. The same procedure was applied to xylene extracts of three low potency oils. The zone of maximum absorption was found to be identical with that of high-potency oils. Transmission by the xylene-chloroform mixture alone, which served as a blank, was 97% in this zone.

Table I. Transmission of Chloroform and Chloroform-Xylene Solutions of the Violet Vitamin A-Antimony Trichloride Color

Wave Length $m\mu$	Transmission	
	Chloroform %	Chloroform-xylene %
500	62.0	63.5
510	55.5	56.5
520	47.25	48.25
530	42.0	43.0
540	39.0	40.0
550	38.0	38.5
560	41.0	41.5
570	53.5	53.5
575	70.0	70.0

#### ANALYTICAL PROCEDURE

A 0.2- to 0.5-gram portion of a representative liver sample which has been previously homogenized for 5 to 8 minutes in Waring Blendor, is transferred quickly to the bottom of a tared 15-ml. graduated conical centrifuge tube and weighed to the nearest milligram. Ten milliliters of purified xylene are directed in a sharp stream to the center of the material in the tube which is shaken vigorously for 50 to 100 strokes, centrifuged for 5 minutes at 2500 r.p.m., inverted once or twice, and recentrifuged for another 5 minutes. The total volumes of liver, solvent, and xylene-insolubles are recorded and a 5-ml. aliquot of the supernatant extract is evaporated to dryness at 65° to 85° C. under atmospheric pressure. The evaporation of the solvent under these conditions is complete in about 4 hours. A 1-ml. aliquot of the supernatant is also collected and is diluted as required for the colorimetric assay. The Rosenthal-Weltner color is developed as mentioned above and its intensity is measured in Klett-Summerson photoelectric colorimeter. Results are presented in Table II.

#### CALCULATION OF RESULTS

The percentage of oil in the liver sample can be easily calculated from the formula:



$$\frac{100 w(V - v)}{5 W} = \frac{20 w(V - v)}{W} = \text{percentage of oil in liver}$$

where  $W$  is the weight of liver,  $w$  is the weight of oil in the 5-ml. aliquot,  $V$  is the total volume of liver and solvent, and  $v$  is the volume of the xylene-insoluble residue.

The potency in International Units per gram of oil is calculated from the following:

$$\frac{R}{V} \times \frac{F}{p} = \text{I.U. per gram of oil}$$

where  $R$  is the colorimeter reading,  $F$  is the conversion factor employed to convert readings to International Units, and has a value of 42.6 as determined on various concentrates,  $V$  is the volume in ml. of undiluted extract, and  $p$  is the percentage of oil in the undiluted extract.

Table II. Comparison of Methylene Chloride and Xylene Extraction Methods for Oil and Vitamin

Sample No.	Xylene-Centrifuge		Methylene Chloride		
	Oil content	Rosenthal-Weltner	Oil content	Ultraviolet absorption	Rosenthal-Weltner
	%	I.U./g. oil	%	I.U./g. oil	I.U./g. oil
C4	50.0	100,700	53.0	128,600	99,100
C5	51.3	96,500	54.5	107,200	91,700
C6	74.0	24,500	74.1	33,000	26,200
119	58.8	167,200	59.8	..	153,100
158	36.5	3,460	39.0	3,180	3,570 <sup>a</sup>
171	75.0	278	70.8	300	..
	75.0	278	..	..	..
	74.7	293	..	..	..
	77.9	285	..	..	..
	72.1	270	..	..	..
172	74.1	53,400	77.3	61,200	54,000
173	75.8	37,700	76.3	41,200	36,500
174	78.4	38,900	76.4	..	39,900
180	78.8	52,000	74.3	..	54,800
181	46.6	4,324	43.3	..	3,280
	47.1	4,468	..	..	..
216	70.1	14,400	68.1	..	15,100
	69.6	14,400	..	..	..
	69.1	13,900	..	..	..
	67.4	13,500	..	..	..
	68.9	13,500	..	..	..
217	37.8	352,200	41.9	..	332,800
	38.2	362,000	..	..	..
	41.0	342,300	..	..	..
	34.2	357,000	..	..	..
	34.1	329,500	..	..	..

<sup>a</sup> Routine application of methylene chloride technique (2) gave a cloudy extract possessed of an orange-red rather than violet color. The sample therefore was saponified and on analysis by the xylene method gave the correct color and a value which agreed closely with that obtained by application of the xylene method to unsaponified oil.

## DISCUSSION

The maximum intensity of the Rosenthal-Weltner color appears at the moment the blue tinge about the longitudinal axis of the tube has disappeared and requires about 0.75 minute to develop. At room temperature the color is stable for 20 to 30 minutes and then slowly decreases in intensity with the appearance of an orange tinge.

Table I indicates that the presence of xylene in the color reaction has no significant effect on either the position of the absorption maximum or the intensity of the color produced. In fact, xylene up to a concentration of 40% in the final reaction mixture has no effect on the color intensity. If, however, chloroform is completely replaced by xylene in the reaction, the value of the conversion factor employed changes.

Table II compares the results obtained with methylene chloride (2) and xylene extraction procedures for oil and vitamin A with a few figures for the potencies obtained by direct absorption at 328 mμ. Samples 171, 216, and 217 were repeated 5 times each in order to test the precision of the method under ordinary routine conditions. The average values for these samples were: 75.0 ± 1.6%, 69.0 ± 1.0%, and 37.0 ± 6.2%, respectively, for the percentage of oil; and 280 ± 2.1%; 13,940 ± 2.1% and 348,500 ± 2.9%, respectively, for the potency.

The agreement between the colorimetric values obtained on xylene or methylene chloride extracts is sometimes excellent and sometimes only fair. An explanation may be found in the fact that even low-potency xylene extracts analyzed by the Rosenthal-Weltner reaction give colors that follow Beer's law in the ranges studied, an observation that is not always realized when using other solvents. The author believes that xylene does not extract certain undesirable products that tend to cause the color to deviate from linearity and to be off shade.

Most of the results obtained on direct absorption at 328 mμ are high compared to the colorimetric values. This observation is hard to explain, but the direct spectrophotometric method, like the biological, is an entirely different method of assay: it is unusual for the three methods to be in close agreement. However, samples C4, C5, and C6 were core samples whose previous histories are not well known, while samples 158, 172, and 173 were fresh samples kept in dry ice until analyzed.

Certain livers of low oil content, such as No. 217, present erratic results. The precision of the method in these cases can be greatly increased by making two extractions with 7 ml. of xylene each, the first containing a single drop of 20% trichloroacetic acid. In such cases the contents of the centrifuge tube are stirred vigorously by means of a spiral conical stirrer made of 0.75-mm. yellow copper alloy spring wire. The combined extracts are made up to 15 ml. with xylene and analyzed in the usual manner. This treatment coagulates the tissue and allows the solvent and tissue to separate freely.

The 4 hours required to evaporate the xylene from the 5-ml. aliquots is an advantage in mass analysis. The loss of solvent during centrifugation and other manipulations is reduced to a minimum and ample time is afforded to assay the potency of a large number of samples before it becomes necessary to weigh the residual oil.

Experiments with kerosene, a very inexpensive and readily procurable solvent, show that it may be used in emergency in place of xylene; however, its use is limited by the low solubility therein of antimony trichloride; this militates against the use of kerosene in analysis of livers of very low potency.

## SUMMARY

Purified xylene may be successfully used to extract oil and vitamin A from shark livers and the potency of the oil may be determined directly on an aliquot of the xylene centrifuge extract by the Rosenthal-Weltner method. The violet color follows Beer's law even with low-potency samples, a fact not always realized when using other solvents for the extraction. The extracts are never cloudy, as is often the case when using such solvents as diethyl ether, chloroform, or methylene chloride. Through the use of purified xylene it is possible to simplify further an already rapid method for the simultaneous determination of oil and vitamin A in these livers.

## ACKNOWLEDGMENT

Grateful acknowledgment is made for the constant assistance extended by J. Murray Luck, who supervised the investigation, and to R. A. Bolomey, whose data for ultraviolet absorption were used in Table II, and who gave much helpful criticism and many excellent suggestions throughout the course of the work.

The investigation itself has been incidental to a survey of the vitamin A potency of soupfin shark livers. The survey is part of a larger study of the entire California soupfin shark fishery, which is being conducted by the California Division of Fish and Game.

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# Colorimetric Determination of Germanium as Molybdigermanic Acid

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A spectrophotometric study of the molybdigermanic acid method for determining germanium shows the procedure works well when the yellow color is developed in 5 *N* acetic acid solution. Preferably the molybdate solution is added to the acidified germanate solution. For 5-cm. thickness the range is 0 to 30 p.p.m., through which

Beer's law applies. Other elements forming colored heteropoly solutions, such as arsenic and silicon, interfere with the determination of germanium by this procedure. Picric acid or buffered potassium dichromate solutions are suitable for use as permanent standards for visual comparison.

GERMANATES, phosphates, silicates, and arsenates have long been known to form heteropoly compounds with molybdates, tungstates, and vanadates. These complexes, of which ammonium molybdiphosphate,  $(\text{NH}_4)_3[\text{P}(\text{Mo}_3\text{O}_{10})_4]\cdot x\text{H}_2\text{O}$ , is a well-known example, have found many applications in analytical chemistry (10). Colorimetric uses are based upon the colors of the soluble complexes or of their reduction products.

Molybdigermanic acid,  $\text{H}_4[\text{Ge}(\text{Mo}_3\text{O}_{10})_4]\cdot x\text{H}_2\text{O}$ , was first prepared by Schwarz and Giese (8) and by Grosseup (3), who noted its intense yellow hue and its solubility in water and certain organic solvents. Although Grosseup suggested its use in colorimetry, Alimarin and Ivanov-Emin (1) were the first to apply it. Several methods based upon reduction of this acid to a molybdenum blue have appeared in the literature since 1930 (2, 4, 6, 7), but little work has been done with any of them.

Since so few have used the acid itself, the present investigation was undertaken to extend our knowledge of the method, special attention being given to determining the optimum conditions for developing and stabilizing the color and to observing the effect of diverse ions upon it.

## APPARATUS AND SOLUTIONS

Transmittancy measurements were made in 5.00-cm. cells with a General Electric recording spectrophotometer adjusted for a spectral band width of 10  $\text{m}\mu$ . All numerical calculations were based on transmittancy readings of the photometer dial at 440  $\text{m}\mu$  rather than on the recorded curve. A glass electrode was used for making pH measurements.

Germanium tetrachloride (c.p.) was hydrolyzed to the dioxide by treatment with water. This material was purified by a procedure similar to that of Johnson and Dennis (5). The standard germanium solution was prepared by fusion of 0.3602 gram of the dry dioxide with 2.0 grams of sodium carbonate, dissolution in water, and dilution to 1000 ml. Dilutions of this stock solution containing 0.1 mg. of germanium per ml. were used.

Ammonium molybdate solutions were made by dissolving the c.p. salt,  $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}\cdot 4\text{H}_2\text{O}$ , in warm water. Since the solutions developed a turbidity on standing, they were prepared fresh every 48 hours.

The various acids used were of the usual reagent quality.

Standard solutions of the recrystallized salts used to observe the effect of diverse ions on the color reaction contained 10 mg. of the ion per ml. of solution. Nitrate, acetate, or sulfate salts of the cations, and potassium, sodium, or ammonium salts of the anions were used.

Recrystallized picric acid, and potassium chromate and dichromate were used in preparing solutions for permanent standards.

Except for storage in Pyrex bottles, no special attempt was made to avoid contamination by silica. Since most of the work was comparative in nature, this should have introduced no serious error. In cases where it was desired to avoid contamination all solutions were used within 24 hours after preparation.

## COLOR REACTION

The yellow color which forms when molybdate solutions are added to acidified germanate solutions depends upon the formation of a heteropoly acid. The intensity and stability of the color are functions of the germanium concentration, the acid

used, its concentration, the molybdate concentration, and the order of mixing the reagents.

Preliminary experiments showed that solutions prepared according to the procedure of Alimarin and Ivanov-Emin (1) faded rapidly. For transmittancy readings made at 440  $\text{m}\mu$  the fading error amounted to 2% in less than a minute after color development. It was obvious that a more stable solution would have to be found to use the method with a photometer.

In order to study the effect of acidity and molybdate concentration on the intensity and stability of the color, 5 ml. of the standard germanium solution were pipetted into a 50-ml. volumetric flask, to this was added a freshly prepared mixture containing known amounts of acid and molybdate, and the solution was then diluted to the mark and mixed. Transmittancy readings were taken at 440  $\text{m}\mu$  at definite time intervals after the color development.

These experiments showed that the intensity of the color developed with sulfuric, nitric, hydrochloric, perchloric, or trichloroacetic acid was extremely sensitive to acid concentration. The maximum color was developed when the solutions were 0.1 to 0.3 *N* in acid, but the exact range depends on the molybdate concentration. Throughout the range of maximum color the solutions fade rapidly. More stable solutions are secured at higher acid concentrations, but at this acidity the intensity of the final color is so sensitive to acid concentration that large errors are produced by extremely small variations in acidity, and the color intensity is much lower than the maximum possible with the system.

Increasing the amount of molybdate in the presence of the strong acids broadens the range for maximum color development, but has little effect on the stability of the system.

The color intensity of solutions prepared with acetic acid increases rapidly until the acidity is about 3.5 *N*. Above this concentration, the color intensity increases slowly. Variations in the molybdate concentration have little effect on the color. The stability of the solutions increases with acidity, solutions more than 2 *N* in acetic acid being stable for about 5 minutes. Solutions more than 6 *N* possess a slight yellow color even in the absence of germanium. The intensity of this color increases with acidity. The optimum acetic acid concentration is about 5 *N*.

Having selected acetic acid as best for the color development the following experimental procedure was used to study the effect of variables on the color reaction. The desired amount of germanate solution, usually 5 ml., was transferred to a 50-ml. volumetric flask, followed by 15 ml. of glacial acetic acid and enough water to make the total about 40 ml. After 5 ml. of 2.5% ammonium molybdate solution had been added, the system was diluted to the mark with water and mixed. Transmittancy measurements were made within 15 minutes after the molybdate addition.

**ACID CONCENTRATION.** The optimum amount of acid was 15 ml. of glacial acetic acid in a final volume of 50 ml. At low

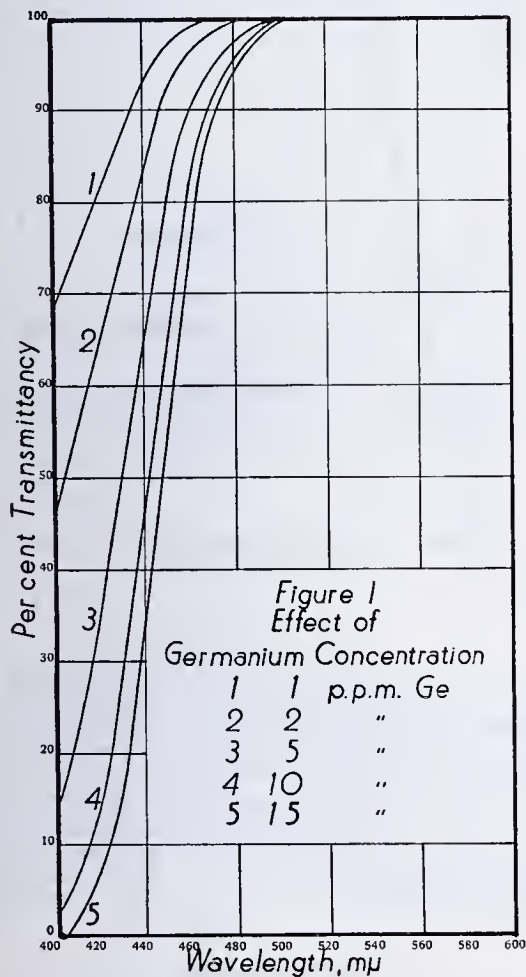


ditions the initial color intensity is not quite so high, and the solution is less stable. At higher acidities considerable color develops in the blank solution, which necessitates a correction. **MOLYBDATE CONCENTRATION.** After enough ammonium molybdate has been added to develop the full color, an excess has little effect. Five milliliters of a 2.5% solution are adequate for amounts of germanium up to 75 p.p.m.

**ORDER OF ADDING REAGENTS.** The greatest stability and intensity of color are obtained by adding the molybdate solution to the acid and then adding this mixture to the germanate solution. Solutions of equal color intensity but of slightly less stability are produced by adding the molybdate solution to the acidified germanate solution. The latter procedure is recommended because of its greater simplicity.

**COLOR STABILITY.** Solutions prepared by adding ammonium molybdate to the acidified germanate solutions fade slowly, the error not exceeding 2% within 15 minutes.

**GERMANIUM CONCENTRATION.** The range of germanium concentration which can be measured with 5-cm. cells is 0 to 75 p.p.m., if transmittancy measurements are made at 440 m $\mu$  (see Figure 1). Beer's law is valid over the entire range. With 1-cm. cells the range is from 1 to 75 p.p.m. of germanium, and the solutions conform to Beer's law up to 40 p.p.m.



**DIVERSE IONS.** Germanium can be separated from most elements by distillation as the tetrachloride. Of the several elements which may volatilize with the germanium, arsenic is the only one capable of forming a colored heteropoly compound. Although it is possible to separate arsenic from germanium by careful distillation, the effect of arsenic on the color development was carefully studied.

With 5 p.p.m. of germanium, the largest permissible concentration of arsenic is 2 p.p.m. In an effort to extend the tolerance for arsenic, various modifications of the procedure were tried. The use of excess ammonium molybdate, as suggested

by Alimarin and Ivanov-Emin (1) or of small amounts of nitric acid in addition to the acetic acid, increases the permissible arsenic concentration. However, in neither case was the increase sufficient to compensate for the resulting loss of stability and reproducibility.

Of the 62 diverse ions studied, the following did not interfere when present in concentrations 100 times that of the germanium: acetate, benzoate, bromide, chlorate, chloride, chlorostannic, citrate, cyanide, formate, iodide, lactate, nitrate, nitrite, oxalate, perchlorate, sulfate, sulfite, thiocyanate, tungstate, bismuth, cadmium, lithium, magnesium, manganese, mercuric, mercurous, potassium, and sodium.

Table I summarizes the data for the ions which interfere, and Figure 2 shows typical transmittancy curves for several solutions containing interfering ions.

Table I. Effect of Diverse Ions

Ion	Added as	Present P.p.m.	Error %	Amount Permissible P.p.m.
AsO <sub>3</sub> ---	NaHAsO <sub>3</sub>	5 (As)	2	5
AsO <sub>4</sub> ---	Na <sub>3</sub> AsO <sub>4</sub>	2 (As)	0	2
B <sub>2</sub> O <sub>7</sub> ---	Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub>	500	4	200
CO <sub>3</sub> ---	Na <sub>2</sub> CO <sub>3</sub>	200	2	200
C <sub>4</sub> H <sub>4</sub> O <sub>6</sub> ---	Na <sub>2</sub> C <sub>4</sub> H <sub>4</sub> O <sub>6</sub>	20	0	20
Cr <sub>2</sub> O <sub>7</sub> ---	K <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub>	50	160	5
F <sup>-</sup>	NaF	50	2	50
PO <sub>4</sub> ---	KH <sub>2</sub> PO <sub>4</sub>	2 (P <sub>2</sub> O <sub>5</sub> )	94	0
P <sub>2</sub> O <sub>7</sub> ---	Na <sub>4</sub> P <sub>2</sub> O <sub>7</sub>	2 (P <sub>2</sub> O <sub>5</sub> )	4	0
S <sub>2</sub> O <sub>3</sub> ---	Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub>	10	0	10
SiO <sub>3</sub> ---	Na <sub>2</sub> SiO <sub>3</sub>	4	24	0
SnCl <sub>4</sub> ---	H <sub>2</sub> SnCl <sub>4</sub>	500	Ppts.	0
VO <sub>3</sub> ---	KVO <sub>3</sub>	2	78	0
Ag <sup>+</sup>	AgNO <sub>3</sub>	500	Ppts.	0
Al <sup>+++</sup>	Al(NO <sub>3</sub> ) <sub>3</sub>	20	14	0
Ba <sup>++</sup>	Ba(NO <sub>3</sub> ) <sub>2</sub>	50	2	50
Be <sup>++</sup>	Be(NO <sub>3</sub> ) <sub>2</sub>	46	2	46
Ca <sup>++</sup>	Ca(NO <sub>3</sub> ) <sub>2</sub>	50	2	50
Ce <sup>+++</sup>	(NH <sub>4</sub> ) <sub>2</sub> Ce(NO <sub>3</sub> ) <sub>6</sub>	500	Ppts.	0
Co <sup>++</sup>	Co(NO <sub>3</sub> ) <sub>2</sub>	50	2	50
Cr <sup>+++</sup>	Cr <sub>2</sub> (SO <sub>4</sub> ) <sub>3</sub>	20	6	5
Cu <sup>++</sup>	CuSO <sub>4</sub>	50	4	25
Fe <sup>++</sup>	Fe(NH <sub>4</sub> ) <sub>2</sub> (SO <sub>4</sub> ) <sub>2</sub>	500	Reduces complex	0
Fe <sup>+++</sup>	Fe(NO <sub>3</sub> ) <sub>3</sub>	50	20	5
Ni <sup>++</sup>	NiSO <sub>4</sub>	100	2	100
Pb <sup>++</sup>	Pb(NO <sub>3</sub> ) <sub>2</sub>	500	Ppts.	0
Sb <sup>+++</sup>	SbCl <sub>3</sub>	500	Ppts.	0
Sr <sup>++</sup>	Sr(NO <sub>3</sub> ) <sub>2</sub>	200	2	200
Th <sup>++++</sup>	Th(NO <sub>3</sub> ) <sub>4</sub>	500	Ppts.	0
Ti <sup>+++</sup>	Ti(SO <sub>4</sub> ) <sub>2</sub>	500	Ppts.	0
UO <sub>2</sub> <sup>++</sup>	UO <sub>2</sub> (C <sub>2</sub> H <sub>3</sub> O <sub>2</sub> ) <sub>2</sub>	20	0	20
Zn <sup>++</sup>	Zn(NO <sub>3</sub> ) <sub>2</sub>	200	2	200
Zr <sup>++++</sup>	Zr(NO <sub>3</sub> ) <sub>4</sub>	500	Ppts.	0

#### PERMANENT STANDARDS

Since the yellow molybdigermanic acid color fades rather rapidly, permanent standards are desirable. Alimarin and Ivanov-Emin (1) proposed solutions of picric acid or potassium chromate for this purpose. Swank and Mellon (9) used buffered solutions of potassium chromate or dichromate for the analogous molybdisilic acid. In order to determine the suitability of these solutions as color standards, and to determine the concentrations equivalent to a definite germanium concentration, a solution of molybdigermanic acid was matched, by means of a Duboscq comparator, with picric acid, potassium chromate, and buffered potassium dichromate solutions. The amounts of the various compounds, in milligrams per liter, equivalent to 10 p.p.m. of germanium were 32.0 for potassium dichromate (buffered with 0.5% borax), 46.4 for potassium chromate (unbuffered), and 4.0 for picric acid. These color matches, checked visually in 30-cm. Nessler tubes, gave the transmittancy curves shown in Figure 3.

The colors of solutions of picric acid or buffered potassium dichromate cannot be differentiated by the eye from molybdigermanic acid, and there is little difference in the transmittancy curves. Unbuffered potassium chromate solutions possess a

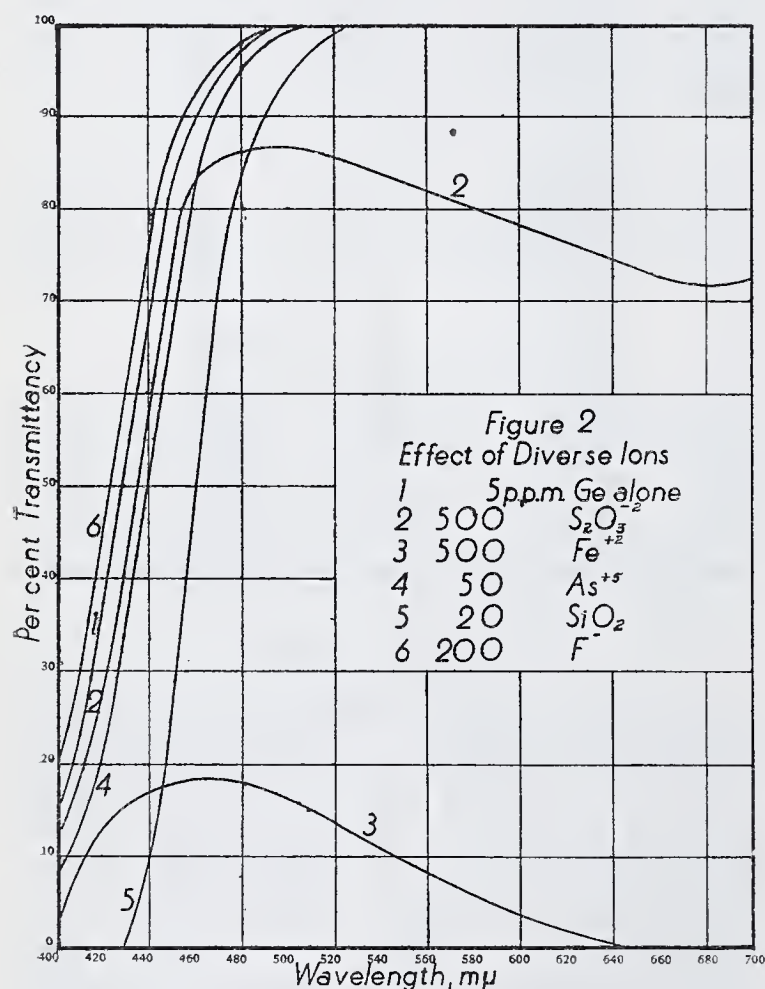


hue different from the other three. As this difference makes accurate visual comparison difficult, these solutions are not recommended for permanent standards. Solutions containing three times the amount of potassium dichromate or picric acid required to match 10 p.p.m. of germanium matched visually and spectrophotometrically a solution containing 30 p.p.m. of germanium. Therefore, either picric acid solutions, or solutions of potassium chromate or dichromate buffered to pH 9, are considered suitable permanent standards.

#### DISCUSSION

The proposed procedure offers a rapid and reliable method for the determination of small amounts of germanium. Its principal disadvantages are the interference of other elements which form colored heteropoly solutions, and the yellow color of the molybdigermanic acid solution. The former necessitates separation of germanium from silicon and arsenic, with which it is commonly associated, before an accurate determination can be made.

Comparison of this work with that of Alimarin and Ivanov-Emin (1) reveals several differences in the results, most of which can be attributed to the greater sensitivity of the photoelectric instrument used in the present study as compared to the earlier visual instrument.



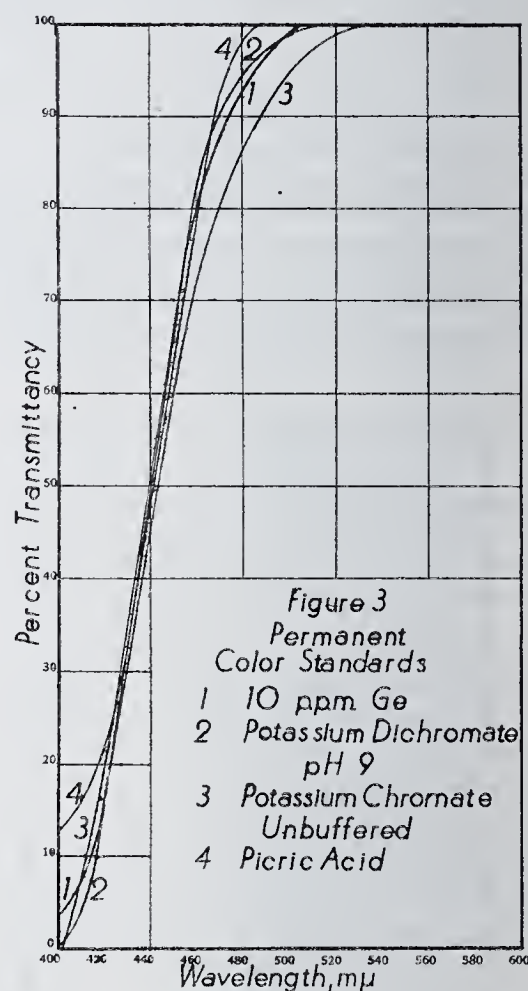
The choice of picric acid or buffered potassium dichromate for permanent standards may be left to the analyst using the method. The dichromate is usually available as a primary oxidimetric standard.

#### RECOMMENDED PROCEDURE

**TREATMENT.** Dissolve the sample by appropriate means, if necessary, and remove or inhibit any interfering ions to bring their concentration within the limits set in Table I. If separation

of the germanium is unnecessary, neutralize the solution with dilute acid or base, dilute to a definite volume, and proceed as described under measurement.

If it is necessary to separate the germanium by distillation the tetrachloride, follow by precipitation as the disulfide from solution 6 N in sulfuric acid. Such sulfide precipitates, after thorough washing, should then be dissolved in the smallest possible amount of distilled aqueous ammonia. Transfer the yellow solution to a platinum dish, decolorize with 30% hydrogen peroxide, and add 1 to 2 ml. excess. Boil the solution gently to destroy excess peroxide, cool, neutralize with dilute sulfuric acid and dilute to a definite volume.



**MEASUREMENT.** Transfer an aliquot of this solution, containing 1 to 3 mg. of germanium, to a 100-ml. volumetric flask. Add 30 ml. of glacial acetic acid, dilute the solution to about 80 ml. with water, and then add 10 ml. of a freshly prepared 2.5% solution of ammonium molybdate. Dilute to the mark, mix well, and measure or compare the color immediately by any of the usual means. Standards for comparison may be prepared similarly, permanent standards containing picric acid or potassium chromate buffered to pH 9 may be used. With permanent standards a blank should be run to correct for silica in the reagents.

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# Improved Fractionating Column for Gases

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The improved automatic fractionating column for low-boiling gases described is of the constant-pressure type and is of greatly improved efficiency and capable of precise temperature control over a wide temperature range at least up to  $10^{\circ}\text{C}$ .

ONE of the difficulties in the smooth operation of automatically controlled fractionating columns for purification of gases with low boiling points, in which the head temperature is maintained by the injection of liquid air, is the lag in the injection period behind the actual need. This results in considerable temperature fluctuations in the head and overcooling at the end of the injection cycle. Booth and Bozarth (1) partially overcame this difficulty by clamping almost shut the liquid air exit tube from the head, so that only a small amount of liquid air went through the head at each injection, but even then at very low temperatures small variations in head temperature were disclosed as a slightly wavy line on the recording potentiometer chart. Such temperature variations interfere with the purification of gases, particularly when the boiling points of the gases to be separated are close.

The column shown in Figure 1 is of the constant-pressure type described by Booth and Bozarth (1), in which the pressure is maintained by controlling the cooling of the still-head condenser by a contacting manometer connected to the still head. Its control of the head temperature is so delicate that liquid air has been used in it as the refrigerant successfully to distill fractionally a gas boiling at  $+8^{\circ}\text{C}$ . without freezing up of the head and with the temperature holding perfectly constant.

## FRACTIONATING COLUMN

**REFLUX CONDENSER.** A sectional view of the still head is shown in Figure 2. The condenser jacket consists of a brass tube 2.5 cm. in diameter and 22 cm. long, the ends of which are turned to a slight taper to accommodate the cork stoppers which close the ends. At the bottom of the condenser jacket, the space between the outer tube of the jacket and the glass tube of the column is filled with a solid brass cylinder 4 cm. long. This solid section of brass serves as a heat reservoir to smooth out fluctuations in the rate of cooling. The liquid air inlet tube opens into a groove in the top of this cylinder. In the condenser jacket, the liquid air is forced to follow a longer path by a spiral baffle,

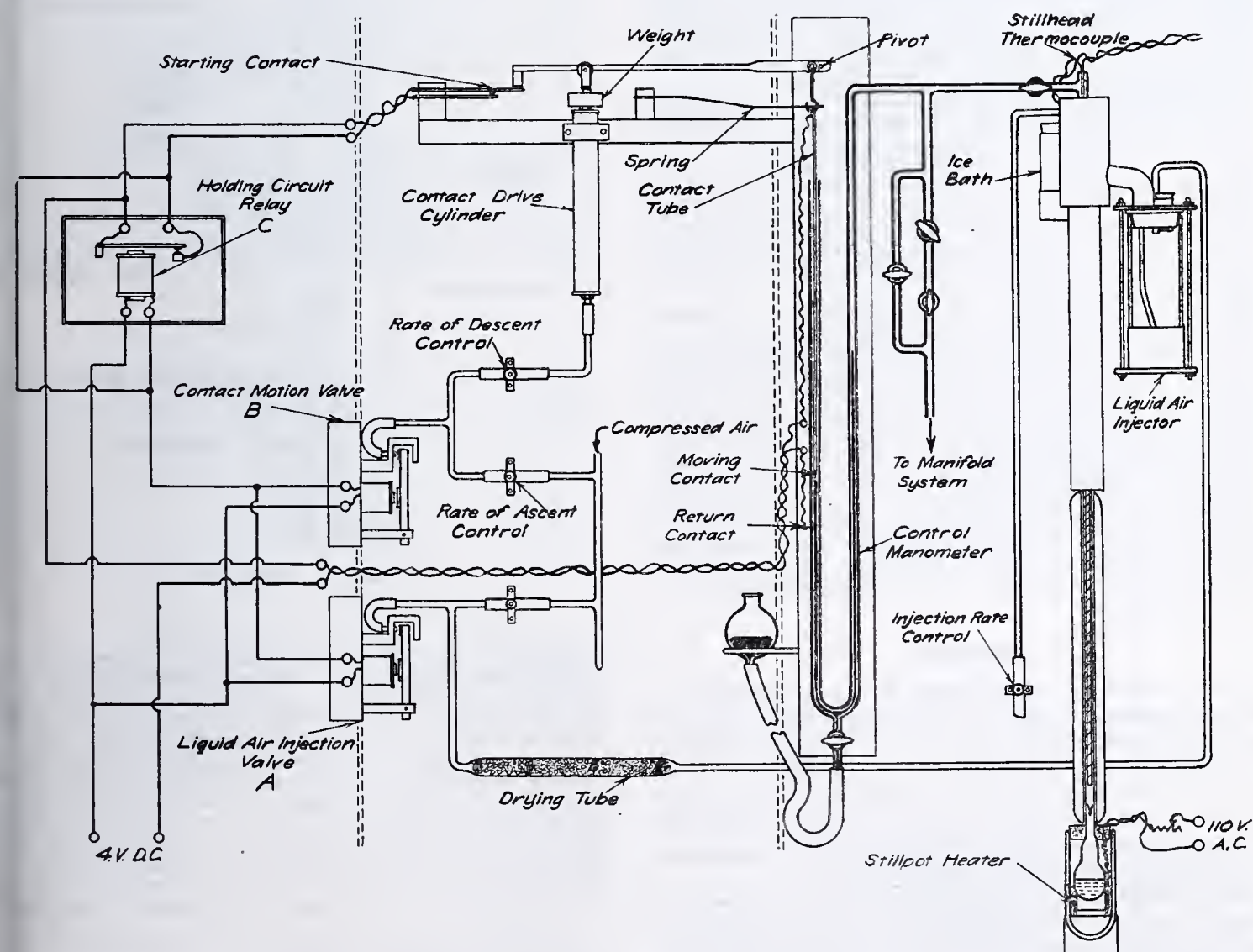


Figure 1. Diagram of Fractionating Column

Left, electrical control center. Center, air-operated control apparatus. Right, distilling column



made by winding No. 6 copper wire around a suitable mandrel and slipping the finished spiral into the jacket. The spiral is securely soldered to the jacket and the center bored out in the lathe to fit snugly over the glass tube. Just above the air outlet, a washer is fastened to the jacket to prevent the cork stopper from being pushed over the air outlet. The corks are compressed to give a tight seal by means of clamping yokes pulled down by tie rods running between the two ends (not shown in drawing). These tie rods are imbedded in the cork insulation. For the first few runs, the nuts on the tie rods must be tightened at the beginning of each run to ensure a tight seal at the end of the condenser jacket.

Since the liquid air comes in direct contact with the glass tube of the still head, rapid response to the control action is obtained and this condenser has a very high cooling capacity for its size.

For gases boiling below  $-50^{\circ}\text{C}.$ , the still head may be cooled with liquid air, using a liquid air injector of the kind described by Booth and Bozarth (1). For cooling the still head in the distillation of higher boiling gases, a dry ice-acetone cooling system may be used, although liquid air has been used successfully up to  $+8^{\circ}\text{C}.$

**RECTIFYING COLUMN.** The column consists of a straight glass tube of 8-mm. inside diameter containing an inner glass tube of 5-mm. outside diameter and 90 cm. long, which is sealed off at both ends. The inner tube is spaced from the outer by means of a wire spiral consisting of one No. 26 Nichrome wire wound around another one. This is made by coiling one piece of Nichrome wire around a mandrel about 1 mm. in diameter, such as a steel knitting needle. The coil is taken off the mandrel and a straight piece of wire is slipped through the center, the two are clamped together at one end, the straight wire is pulled taut and clamped at both ends, and the coil is pulled out until it fits snugly over the straight wire. This is then wound around the inner glass tube in the form of a spiral with a pitch of about 9 mm. This spiral spaces the inner tube from the outer, makes the ascending vapors follow a spiral path up the column and consequently travel a greater distance, increases the surface of contact between vapor and liquid, and gives better distribution of the liquid around the column. Most of the descending liquid runs straight down the surfaces of the tubes, but at each turn the spiral picks up a certain amount of liquid and carries it around to a different point in the column.

At the bottom of the column, just above the still pot, a dropper is located for observation of the rate of reflux and to return the liquid directly to the surface of the liquid in the still pot.

**THERMAL INSULATION.** The still head and upper half of the column are insulated with a layer of cork about 2.5 cm. thick, which is coated with waterproof paint. The lower part of the column is insulated by a vacuum jacket made from a Pyrex condenser, in order to be able to observe the liquid in the column. It can be insulated entirely with cork, with a small peephole to observe refluxing if desired.

**STILL POT.** The body of the still pot is about 4 cm. in diameter and 6 cm. high with a neck 2 cm. in diameter. Clamped against the outer edge of the bottom is an electric heater unit wound on a brass ring. A single layer of thin asbestos tape is put between the metal ring and the glass to prevent direct contact between metal and glass, which might cause breakage of the still pot. The outside of the heater unit is insulated with asbestos to prevent heat from reaching the upper part of the still pot and causing superheating of the vapors passing into the column. To prevent heat radiation from the outside the still pot is insulated by a silvered Dewar flask, the top of which is tightly closed by a cork stopper.

By the use of a larger diameter still pot, more evaporating surface and a shallower body of liquid are obtained, both of which diminish the tendency of the liquid to superheat and produce pressure surges.

## CONTROLS

**SYSTEM OF CONTROL.** This column, like the one described by Booth and Bozarth, is operated at constant pressure, which is maintained by controlling the cooling of the still-head condenser by a contacting manometer connected to the still head. This system of control, shown in Figure 1, operates by injecting liquid air into the still-head condenser when the pressure rises enough to close the manometer contact. This cools the still head, lowers the pressure, and then opens the contact again.

Since the head is cooled intermittently, the use of a column having a low liquid holdup produces a number of difficulties, which require some modification in the method of control. Because of the lag between the control action and the effect as transmitted to the control manometer, the mercury tends to overshoot

the contact point and causes fluctuations in the pressure. This in turn, causes large changes in the rate of flow of reflux, which seriously diminish the efficiency of the column.

To decrease these pressure surges and to check the variation of the flow of reflux, a system of control was devised which minimizes the effect of lag on the response to the control action. A mechanism is employed which gradually lifts the control contact while liquid air is being injected into the still-head jacket, resulting in the interruption of injection at a pressure higher than that at the beginning of injection, and thus shutting off the liquid air ahead of the normal time of cutoff. This "anticipator" device eliminates the excess cooling which otherwise would cause the pressure to drop below the normal cutoff position.

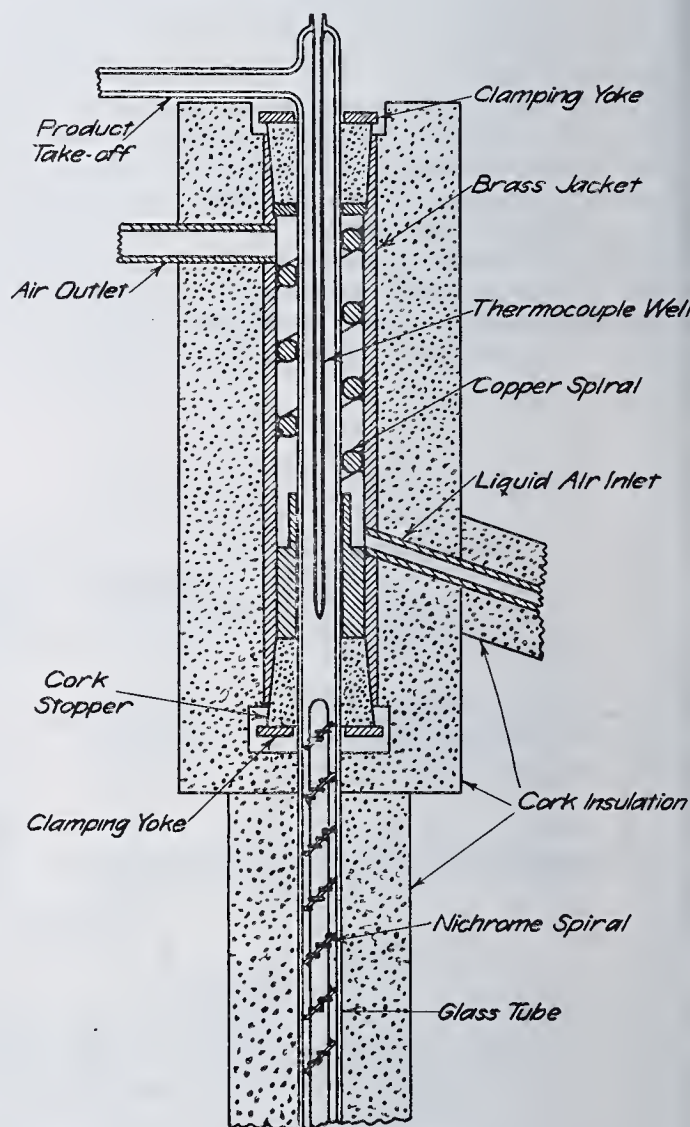


Figure 2. Still Head

As shown in Figure 1, liquid air injection is controlled by a magnetic valve, A, made from a telegraph sounder as described by Booth and Bozarth (1), which closes the leak in the air line leading to the injector. Another magnetic valve, B, controls a leak in the air line leading to the contact drive cylinder shown in Figure 3, made from a small bicycle pump, the piston of which is connected by a lever to the glass tube carrying the control contact. The coils of the two magnetic valves and of the holding circuit relay are connected in parallel, so that all close simultaneously when energized. (The holding circuit relay, C, is a 25 ohm, Type PC, Allied No. 77-070 relay from Allied Control Co. Inc., New York, N. Y.) A pair of electrical contacts, known as the starting contacts, are located on the frame of the contact drive mechanism and close when the piston is in the down position. These contacts are connected in parallel with the main contacts on the holding circuit relay.

The 4-volt direct current power supply to the control circuit passes first through the manometer contacts, then through



starting and holding contacts, and fully through the relay and magnetic valve coils.

In operation, when the control manometer contact closes, the magnetic valves and relay close, injecting liquid air into the still head, and slowly lifting the control contact. Though the starting contact is opened as soon as the piston starts upward, the holding relay contact parallel with it is still closed, so that current continues to flow through the valve coils until the manometer contact opens again and releases the valves and relay. After this, the electrical circuit cannot be completed again until the piston reaches the down position, even though the manometer contact might have closed, because the circuit cannot be completed until the starting contact closes.

The drive piston is connected by a lever and linkage to the tube carrying the movable contact in such a way that a large movement of the piston produces a small movement of the contact. A 200-gram lead weight is carried on the piston rod to return the piston to the down position, and a stiff spring connected to the top of the contact tube keeps the system of linkages in tension and thus eliminates lost motion. The stop on the contact drive piston (not shown) which restricts its upward motion is set to allow the movable contact to be lifted about 3 mm. The control contact consists of a platinum tip soldered to a copper lead wire and sealed with de Khotinsky cement into the end of a glass tube 2.0 mm. in diameter to give stiffness to the contact wire. A fixed platinum contact is sealed through the manometer tube below the surface of the mercury to complete the circuit through the mercury.

Regulation of the speed of rise and descent of the control contact is accomplished by adjusting the screw clamps in the line from the air supply to the magnetic valve and in the line from the valve to the contact drive cylinder.

The method of delaying injection of liquid air until the moving manometer contact has reached the down position was adopted to improve the stability of the system and to prevent chattering of the controls.

### OPERATION

**OPERATION OF THE COLUMN.** First, the gas to be purified is condensed in the still pot with liquid air and, after transfer, any noncondensable gases in the system are pumped out. The Dewar liquid air is removed and the heating coil is put in place under the still pot after the coil has been immersed in liquid air for a moment to avoid sudden warming of the still pot. Then an empty silvered Dewar flask is placed around the still pot and the current is turned on in the injector circuit. While the pressure in the column is rising, the heating Dewar flask is filled with liquid air which is injected into the still head manually before the operating pressure is reached. This cools the still head and column more quickly. After the operating pressure has been reached, the contacting manometer holds it constant.

When the column has been operating for a short time, the contact drive mechanism is adjusted to give the best compensation for the response lag of the system. The rate of ascent and descent of the drive piston and the rate of liquid air injection are adjusted so that the total movement of the contact point is between 1 and 2 mm. The pause between the return of the drive piston to the down position and the start of the next injection is about a half second in duration. If the injection period, which is normally about 2 or 3 seconds, becomes too long and the control contact rises too far, the rate of liquid air injection should be cut down. When properly adjusted, this control system can hold the pressure within 1 or 2 mm. of the control point, even during change of components in the still head, when the pressure is subject to wide fluctuations with the usual method of control.

This column will handle a low-boiling gas like tetrafluoromethane easily, while previous types have been so unstable that operation with such a compound was very difficult.

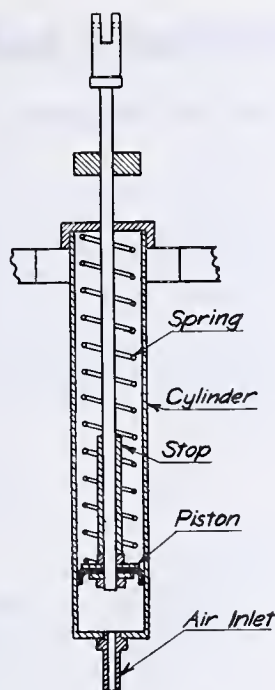


Figure 3. Contact Drive Cylinder

In all other respects, the operation of this column is almost the same as that described by Booth and Bozarth. This apparatus is intended primarily for purification of a single gas, so that an automatic stopcock is not used to control the take-off from the column because it is unnecessary. With a mixture of gases, the automatic stopcock described by Booth and Bozarth should be used. Exhaustion of a fraction from the column was indicated by a slight rise in the time-temperature curve of the still-head thermocouple, indicating the presence of a higher boiling fraction in the still head. With the automatic stopcock this would be indicated by increasingly infrequent operation of the stopcock.

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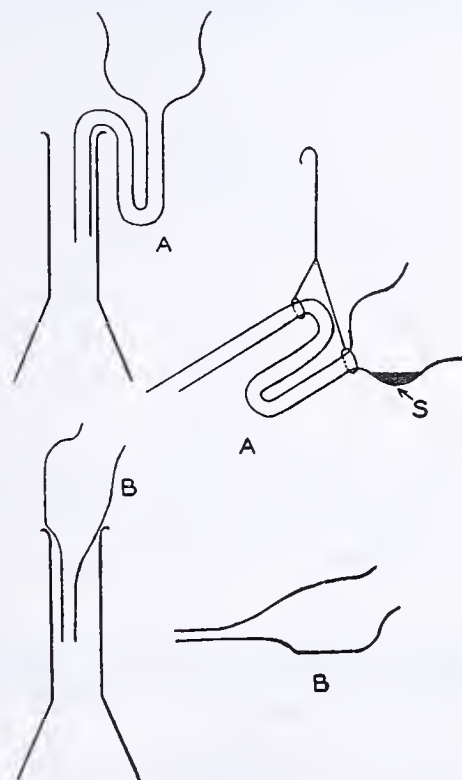
## Weighing Funnels

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**T**WO types of easily constructed weighing funnels have been used in this laboratory to facilitate the accurate weighing and quantitative transfer of solids or liquids to volumetric flasks or other containers for analytical determinations.

In the accompanying sketch funnel A is suspended from the balance hook during the weighing by a wire attached to the S-shaped stem by twisting, the solid or liquid sample is placed in the shoulder, S, of the funnel, and the exact weight of sample desired is adjusted. The funnel is then placed as shown at the left, and the sample is washed quantitatively into the flask by a stream of solvent from a wash bottle. The intermittent siphoning action facilitates the washing of solids into the receptacle.



Funnel B is made with a flat bottom for placement on the balance pan. After the sample is weighed, the funnel is tilted and the sample washed into a flask with an appropriate solvent.

Either funnel may be fitted with ground-glass caps if desired. The diameter of A which the authors have used is 2.5 cm. and the over-all length is 7 cm. The diameter of B is 2 cm. and the over-all length is 6 cm. The shape and dimensions may be modified to meet the requirements of the analyses to be performed.



# Electric Heater for Microprocedures and Melting Points

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Various microprocedures have been worked out for use with the electric heater described. The drying of precipitates, the heating of substances at constant temperatures over long periods, and the like may also be carried out on this hot plate. A wide range of controllable temperatures is obtained.

**A** THERMALLY controlled electric heater of simple and economical construction is described. It has been used by the author for carrying out microprocedures including boiling points (3, 6), extractions (1), fractionations (4, 6), sublimations (3, 5, 7), and sealed capillary reactions (3), and also for melting point determination (2, 8). A wide range of controllable temperatures is obtainable.

## APPARATUS

An electric flatiron of 5-ampere capacity and 550-watt rating is used in the preparation of the heating block. Two holes of 8-mm. diameter are drilled diagonally through the base (11 mm. thick), so that they meet at the center of the block (Figure 1). One hole is for a 360° thermometer (Figure 2) and the other is for a microreaction capillary tube. Several other small-diameter holes which need not terminate in a central point, are drilled through the base for holding tubes to be used for sublimations, sealed capillary reactions, and the like.

A variable-voltage transformer or a rheostat of 5-ampere capacity may be used for thermal control, but lamp bank control has given the author very satisfactory results. For finer temperature control, a slide-wire rheostat may be used in conjunction with the lamp bank. Figure 3 shows the current input necessary for obtaining a convenient temperature range. By increasing the current input, proportionately higher temperatures will be obtained. If desired, time-temperature and constant gradient calibration curves may be obtained by the method described by Dowzard and Russo (2).

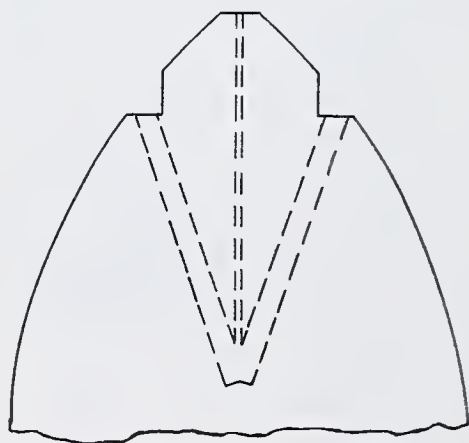


Figure 1. Top View of Heater

A short preliminary working acquaintance suffices to establish familiarity with the arrangements necessary for satisfactory operation under various demands.

## PROCEDURE

The procedure for taking melting points is essentially as described by Shriner and Fuson (8). Small amounts of the substance are deposited on the hot plate throughout small interval rises of temperature. The moment an instantaneous fusing is observed, the temperature is read and taken as the melting point of the substance. Glass wool is used for wiping off the surface of the heater.

In taking the melting point, the area over the thermometer bulb is used, because noticeable temperature differences arise

between the center and the outer regions of the block when the temperature rises above 100° to 125° C. To determine the area of uniform temperature range, a solution of a substance possessing a conveniently low melting point and low volatility at temperatures near the melting point—e.g., urea—is spread on the surface of the plate and evaporated by raising the temperature of the plate somewhat. A thin coating of the substance remains on the surface. Now the temperature is raised rapidly until within a few degrees of the melting point, after which

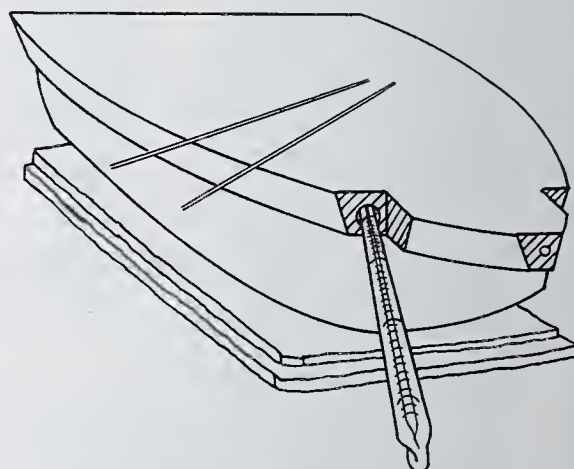


Figure 2. Thermometer and Boiling Point Capillaries in Position in Heater

heating is adjusted to give a rise of about 1° per minute. The areas within which the substance melts for 0.5° or 1.0° intervals are noted, these are the demarcation areas of uniform temperature range and give an indication of the best distribution over surface. This process may be repeated with other substances of different melting points.

For determining boiling points (3, 6) the author has found the following procedure satisfactory:

A narrow capillary (0.3-mm. internal diameter and 7 to 8 cm. long) is used. No tapered stem is required. Liquid is drawn to a column of 5 mm. or more, then the capillary is sealed in the usual way. Care should be taken not to make the sealing too large; otherwise, proper contact of the whole length of

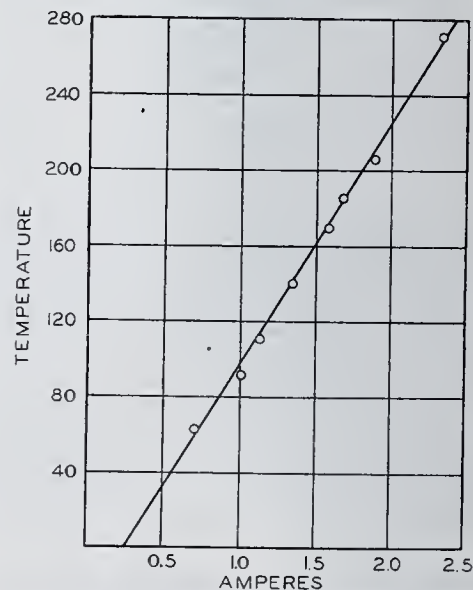


Figure 3. Temperature-Ampere Characteristics of Heater



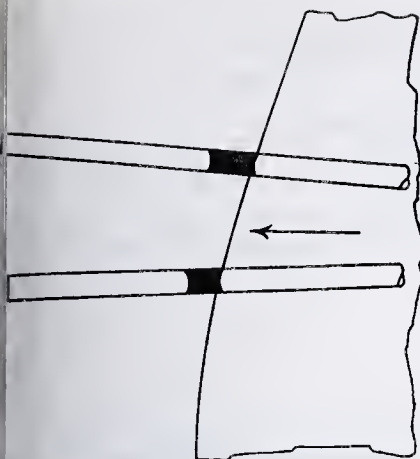


Figure 4. Position of Liquid in Capillary at Microboiling Point

capillary with the surface of the plate not be effected. The capillary is so placed that the sealed end is approximately at the center of the block and the open end projects about 2 cm. off the plate (Figure 2).

When approaching the boiling point, the liquid starts moving restlessly back and forth in the capillary. When the boiling point is reached, the liquid remains stationary, and the lower meniscus coincides with the edge of the block (Figure 4). Boiling of the block causes the liquid to be drawn into the capillary, and a repetition of the boiling point determination is possible.

For carrying out microextractions, an extraction tube having a small bulb at each end is fashioned (Figure 5). The material to be extracted is introduced into the capillary, which is tapped lightly to shake all the material into the lower bulb. If any material adheres to the walls of the capillary, a small wad of glass wool is used to push it down into the bulb. Next, an "internal" filter of powdered glass wool or asbestos is prepared. When the capillary has cooled, the solvent is introduced into the tube and centrifuged to the lower bulb. Enough solvent is used to fill the bulb about two thirds, and is cooled by submerging the lower bulb in an ice bath. When the solvent has cooled sufficiently, the upper end of the capillary is sealed.

For carrying out the extraction, the heater is maintained at a constant temperature, which should be about equal to that of the boiling point of the solvent. When the heater has reached the required temperature, the capillary tube is laid horizontally on the surface of the heater and allowed to heat for several minutes, so that maximum solution of the substance is effected. When the hot solution is centrifuged to the other bulb. A small quantity of liquid is allowed to remain in the stem on the side of the empty bulb, so that a vapor equilibrium will be effected, thus preventing the tendency of the solvent to go over to the empty bulb during heating. The bulb containing the solution is submerged in the ice bath and kept there until maximum precipitation has occurred. Then the liquid is recentrifuged to the other bulb, and the operations are repeated until the extraction is complete.

The completeness of extraction is conveniently determined by use of the polarizing microscope (1). To prepare the extraction tube so that it may be examined under the microscope, it is necessary to flatten a portion of the tube on the same side as the "precipitation" bulb. During the extraction procedure, some solution should be allowed to remain in the flattened area, so that crystals will deposit in this area. These crystals may then be examined for homogeneity and other characteristics that may be required to establish their identity.

For microsyntheses, tubes similar to the one described for microextraction may be used. The methods used in introducing the reactants and in preparing the internal filter are the same as for microextractions. After the reaction has been completed, the empty end of the tube is opened, and solvent (charcoal is also added if decolorization is necessary) is introduced and centrifuged to the bulb containing the reaction mixture. After

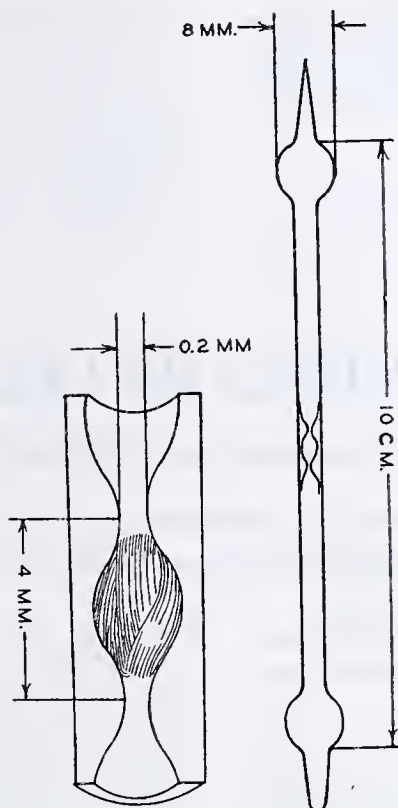


Figure 5. Microextracting Tube

the solvent has been cooled the tube is resealed and heated until solution is complete. Then the solution is centrifuged to the other bulb and allowed to cool until crystallization is complete.

For sublimations, tubes similar to those used in microfractionations may be used. If only minute amounts of substances are to be sublimed, tubes 1 or 2 mm. in diameter may be heated in the small-diameter holes of the heater. For fractional sublimation (7), a long tube (about 10 cm.) is used. A little powdered glass wool in the tubes holds back unsublimable impurities. Hubacher (5) illustrates clearly the advantages and limitations of vacuum sublimation and believes this method "is worthy of greater consideration", a belief which is strongly shared by the author of this paper.

#### DISCUSSION

Dowzard and Russo (2) emphasize the necessity for maintaining a  $0.5^\circ$  per minute temperature rise in melting point determinations, when within  $3^\circ$  or  $4^\circ$  of the supposed melting point. A glance at Table I shows that the heating characteristic of this heater is such that a slow temperature rise is imperative for the most accurate results. The results in Table I indicate that, if the rate of heating is greater than about  $1^\circ$  per minute, the heating of the thermometer lags significantly behind that of the heater's surface.

A comparison was made of the melting points obtained by this block and of an oil bath (Table II). A uniform agreement is noted for the melting points obtained by the two methods.

It is suggested that the experimenter carry out some preliminary boiling point determinations, using capillaries of various sizes (0.1 to, say, 0.7 mm.) to acquaint himself with the

Table I. Effect of Heating Rate on Lag of Thermometer Temperature

(Using m.p. of urea as surface temperature indicator)

Temperature Rise $^\circ\text{C./min.}$	Melting Point (Urea) $^\circ\text{C.}$
0.2	132.7
0.9	132.4
2.5	131.5
5.0	130
13	128

Table II. Comparison of Melting Points Obtained by Electric Heater and Oil Bath

Substance	Melting Point	
	Heater $^\circ\text{C.}$	Oil bath $^\circ\text{C.}$
Urea	132.5	132.5-133
Anhydracetone benzil	147	147-8
Benzimidazole	168	168-9
p-Aminobenzoic acid	183.5	183.5-184
2-Acetyl amino-1, 4-naphthoquinone	201	201-2
Fluorescein	320	—

Table III. Boiling Point Values Obtained with 0.3-Mm. Bore Capillaries

Liquid	Observed Boiling Point ( $\pm 0.2^\circ\text{C.}$ )	
	$^\circ\text{C.}$	
tert-Butyl chloride	50.0	
Methanol	64.5	
Ethanol	78.0	
Aniline	183.0	
Tetralin	207.0	



proper size of tube required for most accurate results. It has been the author's experience that, when capillaries of less than 0.3-mm. internal diameter are used, the boiling point is too low; with larger capillaries, the boiling point is too high (Table III).

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## A DIALYSIS CELL

### For Rapid Quantitative Analytical Determination of Diffusible Components in Blood Plasma

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A simple dialysis apparatus is described which in 2 to 3 hours provides quantitative equilibrium of diffusible constituents in the system and a dialyzate convenient for analyses.

THE dialysis cell here described has been of use in a variety of procedures. The technique is especially applicable when it is desired to prepare a protein-free solution of dialyzable components with any one or a combination of the following conditions: minimal dilution, absence of foreign ions, or exposure to acid or alkaline reagents or to reagents capable of denaturing protein. The apparatus has proved so simple and effective that it can be recommended for general use in analytical dialysis.

#### APPARATUS

The dialysis cell consists of a small wide-mouthed bottle of about 120-cc. capacity, closed by a No. 8 stopper, through which passes a straight glass tube of 28-mm. outside diameter and 11 cm. long. The central tube is closed at its lower end by a cellophane diaphragm held securely in place by many turns of an elastic band, and at the top by a No. 5 rubber stopper. Both rubber stoppers have intravenous needles (No. 18) passing through to allow equilibration of pressure with the atmosphere without loss of liquid by evaporation. The apparatus is assembled as shown in Figure 1.

The technique is like that employed by Hamilton and Van Slyke (1). Two cubic centimeters of plasma were pipetted into the central tube, a glass marble was introduced to give mechanical stirring as described by Northrup and Kunitz (3), and the central stopper was set in place. Eleven cubic centimeters of distilled water were pipetted into the bottle, and the central tube was pushed into place with glycerol as lubricant, and pushed down till the diaphragm was within 1 or 2 mm. of the bottom of the bottle. The bottle was then gently rocked for 2.5 hours, by which time all diffusible amino acids had become uniformly distributed throughout the total 13 cc. of liquid in the system. A convenient rocking device is depicted in Figure 2. Each assembled cell is secured to a narrow board by a stout elastic band. The board is rocked back and forth by a windshield wiper motor, as described by Kunitz and Simms (2).

There are several points to note in the operation of the cell. The diaphragm is tested for leaks by immersing it in water and blowing down the open end of the tube; the absence of any bubbles indi-

cates that the diaphragm is intact and secure. Sausage casing (27/32) has proved satisfactory (supplied by the Visking Corp., 6722 West 65th St., Chicago, Ill.). Some cellophane products are, however, impermeable to water and of course useless for this purpose. A simple test of permeability is to introduce a measured volume of 0.1 N hydrochloric acid into the central tube and dialyze against 2 volumes of distilled water. If the intact membrane is permeable, at equilibrium the concentration of acid throughout will be 0.0333 N.

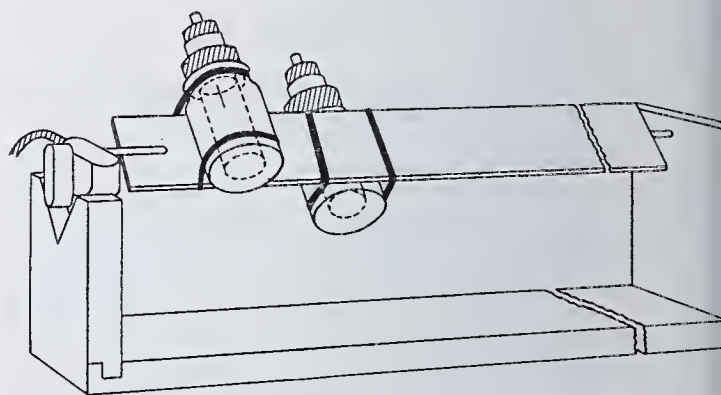


Figure 2. Rocking Device for Dialysis Cell

If the cell is used to prepare dialyzates suitable for ultraviolet spectrographic analysis it is essential to wash the membrane free of substances that absorb light in the ultraviolet. The blanks are easily reduced to low constant amounts by cleaning the membranes in four washes of 100 cc. each of distilled water; each wash is continued for 3 hours with gentle rocking.

For exact quantitative work it is necessary to avoid isolation of droplets on the glass walls. This may be accomplished by having the glass parts, especially the central tube, scrupulously clean and free from grease, or the central tube may be coated within and without by a layer of paraffin. In this latter case the glass marble provides the wettable surface on which to drain the pipet.

In this apparatus a shift of fluid across the membrane in either direction does not influence the final concentration of a freely diffusible constituent (one independent of a Donnan equilibrium), since its concentration will be equal on both sides of the membrane.

The length of time a cell has to be rocked, in order to achieve equilibrium of diffusible amino acids throughout the total fluid volume, was established by dialyzing 2 cc. of plasma against 11 cc. of water. Cells were rocked for 15, 30, 45, 60, 90, 120, 150, 180, and 310 minutes and the dialyzate was analyzed for free alpha-amino acids by the ninhydrin-carbon dioxide method (1). All analyses were in duplicate. Figure 3 shows that equilibrium across the membrane with respect to diffusible alpha-amino acids was achieved within 2 hours of rocking.

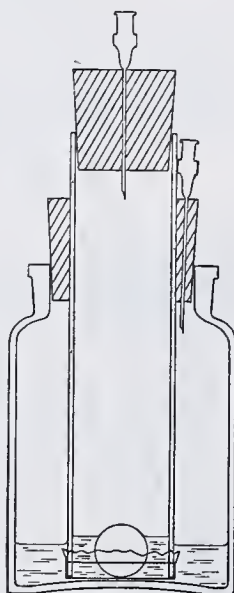


Figure 1. Dialysis Cell



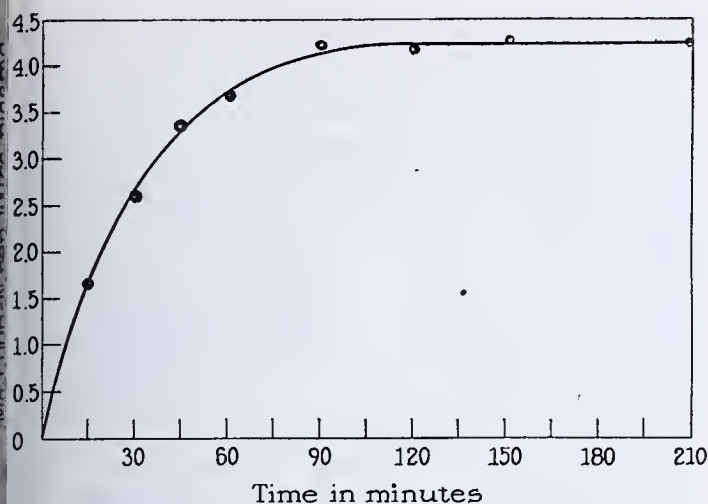


Figure 3. Time of Rocking

Maximum constant value obtained after 2 hours' dialysis indicates equilibrium of diffusible amino acids

Table I. Free Amino Acids

Dialyzate	Alpha-Amino Acid Nitrogen Mg./100 cc. plasma
1	4.64
2	4.71
3	4.66
Av.	4.67
Picric acid filtrate	4.67

The use of the dialysis cell in quantitative analytical analysis demonstrated by the following experiments:

To illustrate the precise quantitative analytical data that can be obtained by means of the apparatus, three separate dialyses were set up with 2-cc. portions of human plasma dialyzed

2.5 hours against 11 cc. of distilled water. Free amino acids were determined on aliquot portions of the dialyzate by the ninhydrin reaction (1) and were compared with amino acid concentrations found by analyses of plasma filtrates obtained by protein precipitation with picric acid (1) (see Table I).

With a plasma-water ratio of 2 to 11 a 5-cc. aliquot of the dialyzate contains the amino acids from 0.77 cc. of plasma. If, however, a more concentrated dialyzate is required, the ratio of plasma to water could be made 12 to 6, so that a 5-cc. aliquot of the dialyzate would contain amino acids from 3.33 cc. of plasma.

Another experiment exemplifies separation of free amino acids from egg albumin.

Table II. Amino Acids in Egg Albumin

	Alpha-Amino Acid Nitrogen Mg./100 cc. plasma
From analysis of dialyzate of egg albumin plus added amino acids	2.88
From analysis of dialyzate egg albumin	0.51
Added amino acid N by difference	2.37
Amount of added amino acid N	2.44

To a 7% egg albumin solution was added an amino acid mixture obtained by the hydrolysis of edestin with strong hydrochloric acid. The amount of amino acid alpha-nitrogen added, calculated from results of a ninhydrin-carbon dioxide analysis on the hydrolyzate (4), was 2.44 mg. per 100 cc. After 2.5 hours of dialysis aliquot portions of the dialyzate were analyzed (Table II).

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## Determination of Small Amounts of Molybdenum in Plants and Soils

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Spectrographic, colorimetric, and polarographic procedures for the determination of small amounts of molybdenum in plants and soils have been studied. It is concluded that, for the ordinary laboratory, the colorimetric procedure is superior if reasonable amounts of sample (1 gram or more of soils, 10 grams or more of air-dried plant material) are available. However, if only small amounts of sample are available (100 mg. of soil, 1 gram of air-dried plant material), the spectrographic procedure is recommended. The polarographic procedure has no particular advantages over the other two.

SEVERAL recent papers (1, 4, 20) have indicated that molybdenum will need to be considered in future studies on the role of various elements in plant and animal nutrition. In one case (1) it was thought to be essential for plant growth; in another case (4) excessive quantities had a deleterious effect on cattle.

The work reported here was undertaken as a result of two observations on the occurrence of this element in plants and soils. In one study (16) it was found that many mineral soils of Florida contained no spectrographically detectable molybdenum. In another study, certain Florida muck soils and some plants grown thereon showed readily detectable quantities of this element (unpublished data). These data made it desirable to study the methods of analysis for very small amounts

of molybdenum with respect to their precision, sensitivity, and other factors.

The methods for the quantitative determination of molybdenum include gravimetric, volumetric, spectrographic, colorimetric, and polarographic procedures. The three latter methods should be more suitable for the determination of very small amounts.

Spectrographically, molybdenum may be determined by a variety of procedures, but there is a growing tendency among workers in this country to use microphotometric methods, with either an internal standard line of the matrix material or an added line standard. The essential feature of this latter method is the introduction into the sample in constant known amounts of an element not originally present. This added element furnishes spectrum lines of constant intensity which, measured with a microphotometer in comparison to the line intensities of the unknown element, give a method of determination.

Colorimetrically, molybdenum is most often determined by the yellow-amber color of its thiocyanate, either directly in the original solution or by first extracting with an organic solvent, immiscible with water (6, 15). Several reducing agents have been used, but stannous chloride has been employed more often than the others. The reaction is affected by several complicat-



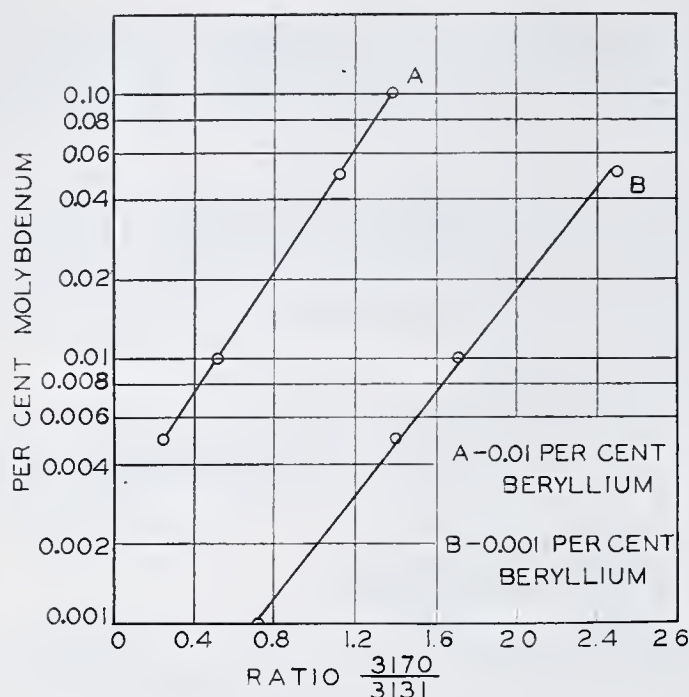


Figure 1. Soil Working Curve

ing factors, including the interference due to the pink complex formation of the thiocyanate ion (which fades rapidly), the fading of the molybdenum complex itself, and the effect of the concentration of hydrochloric acid and other electrolytes on the equilibrium (5, 6, 17). To overcome these effects, most authors have specified strict adherence to a definite procedure and to certain concentrations of reagents which have been found satisfactory.

The polarographic determination of molybdenum has been studied by Uhl (22), Stackelburg (18), Thanheiser and Willems (21), Kanevsky and Shvartsburd (8), and Hokhshtein (7). No satisfactory curves were obtained by these workers in neutral or alkaline solution but phosphoric, sulfuric, or nitric acid solutions were used successfully. Uhl used a nitric acid-ammonium nitrate solution in which the effect of the nitrate and hydrogen-ion concentration on the step height was overcome by the addition of lactic acid.

#### SPECTROGRAPHIC METHOD

A quartz Littrow spectrograph and a nonrecording microphotometer were used. The spectrograph had a linear dispersion of about 5 Å. per mm. at 3200 Å.

Commercial ammonium molybdate was analyzed spectrographically and found to contain traces of calcium, copper, and iron but no zinc, strontium, lithium, cerium, barium, vanadium, yttrium, chromium, manganese, tungsten, aluminium, nickel, zirconium, silver, cadmium, titanium, beryllium, bismuth, antimony, arsenic, tin, or lead. A solution of this ammonium molybdate was prepared and standardized by precipitation as lead molybdate. The solution after standardization was diluted to 0.1 mg. of molybdenum per ml. and other dilutions were prepared only as needed, since it has been stated that 0.001% molybdenum solutions show only 0.2 to 0.4 of their original strength after long storage in glass, owing to base-exchange and adsorption (11). A beryllium solution was prepared by dissolving 5.185 grams of beryllium nitrate trihydrate in water and diluting to 250 ml. This solution contained approximately 1 mg. of beryllium per ml. It was not standardized, but measured amounts of the same solution were used throughout this study. Dilutions of 1 to 10 and 1 to 100 were made to facilitate the addition of small quantities.

Two types of base material for soil analysis were used: silica (powdered sand) and a thoroughly ground and homogenized "synthetic soil", each gram of which contained 0.7 gram of the above silica and 0.1 gram each of ferric oxide, aluminium oxide, and calcium carbonate. Both were analyzed spectrographically for molybdenum and beryllium and none was detected.

Spectrographic standards were prepared from both base materials by adding the molybdenum and beryllium solutions, dry-

ing, grinding, and mixing thoroughly to ensure homogeneity. Five standards containing from 0.1 to 0.001% of molybdenum and each containing 0.01% of beryllium were thus prepared.

Spectrograms of these standards were prepared using 0.7 cm. ( $\frac{5}{16}$ -inch) graphite electrodes which had been cut and bored and purified by the procedure of Standen and Kovach (19), and burned for 2 minutes. Five to 7 mg. of the standard were put in the cup of the lower electrode with a small, half-cylinder-shaped platinum spatula. The standard was not weighed, since, with little practice, this amount can be estimated with some facility and an exact amount is not necessary, since the line standards had been added in known concentration. This approximate weight served as a control on the time of burning, etc. Using a 15-micron slit and Type III-0 Eastman spectroscopic plates (direct current, 250 volts, 12 amperes) was struck (the shutter being opened just before striking the arc) and maintained until the sample was completely volatilized. The arc was focused on the slit with a quartz condensing lens and arc wandering was corrected manually. After processing, the galvanometer deflection ratios of the molybdenum 3170 Å. and beryllium 3131 Å. lines were determined with the microphotometer. The average values thus obtained were used to plot the working curves in Figure 1.

Working curves for plant materials (Figure 2) were prepared in a similar manner, except that since the matrix material of plant ash is different from soils, a base material was made up so that each gram contained 0.806 gram of calcium carbonate, 0.030 gram of magnesium oxide, 0.117 gram of potassium sulfate, 0.018 gram of sodium chloride, and 0.029 gram silicic acid. All the materials were found spectrographically to be free from beryllium and molybdenum.

Ten samples of plants and soils were analyzed in the following manner. The material was dried at 110° C., then ashed at 450° (at 550° C. there is danger of loss of molybdenum by volatilization), carefully pulverized, and mixed in an agate mortar. A 1-gram portion of the ash was weighed out (100 mg. would be sufficient), sufficient beryllium solution was added to give 0.001% of beryllium, and the material was dried and again homogenized. Quintuplicate spectrograms were prepared and measured with the microphotometer, and the proportion of molybdenum determined from the appropriate curve. The results of the analysis are given in column 3 of Table I.

Table I. Analysis of Soils and Plants

Nature of Sample	Ash %	Molybdenum <sup>a</sup>		
		Spectrographic %	Colorimetric %	Polarographic %
Woody peat (0-9 inches)	22.84	Trace (below calibration)	0.00065	0.00065
Brighton peat (0-6 inches)	52.95 <sup>b</sup>	None	0.0003	Trace
Brighton peat (6-18 inches)	8.81	0.0015	0.0011	0.0011
Everglades peat (0-8 inches)	11.41	0.0026	0.0021	0.0021
Okeechobee muck (8-20 inches)	48.63	0.0022	0.0019	0.0019
Dallis grass	10.25	0.0035	0.0031	0.0031
Para grass	5.07	0.037	0.032	0.032
Napier grass	13.04	0.0057	0.0042	0.0042
Sugar cane leaves	4.47	0.0028	0.0023	0.0023
Saw grass	3.34	0.0031	0.0025	0.0025

<sup>a</sup> On ash basis.

<sup>b</sup> Probably due to previous burning.

Neglecting the time for ashing, preparation of the sample requires about 30 minutes, and the additional time required for each determination on a routine basis is estimated at about 10 minutes. There is also the possibility of simultaneous determination of several elements, which would decrease the time per determination.

The factors affecting the precision of this procedure (1) nonuniformity of the sample, (2) contamination, (3) variation of exposure conditions such as wandering of the arc and change in line voltage, and (4) the photometric error. Two replicate analyses of the sample of sugar cane leaves showed a probable error of a single determination of approximately 1% of the mean. The use of solutions of both the standards and unknowns might possibly increase the precision, but would increase the time required for analysis and the danger of contamination.



The factors affecting the accuracy are (1) incomplete burning (2) the influence of varying major constituents on the volatility and excitability of the molybdenum and beryllium atoms. In this work the sample was always completely burned and standards approximating the composition of the samples were used. The lower limit of detectability of molybdenum depends to some extent on the material being analyzed. The absolute sensitivity of the 3170 Å. line appears to be about 0.05 microgram under the conditions used here; hence, with a 10-mg. sample approximately 0.0005% of molybdenum could be detected. Chemical concentration could increase the sensitivity, but would partially vitiate the advantages of a spectrographic method.

#### COLORIMETRIC METHOD

Certain features of Sandell's procedure (17) have been combined with some of those of Hoffman and Lundell (6) in this work. A solution of hexavalent molybdenum in a volume of 50 ml. was prepared, to which were added 7 ml. of concentrated hydrochloric acid. To this solution, contained in a separatory funnel, 3 ml. of 10% potassium thiocyanate solution and 3 ml. of stannous chloride solution (10 grams of stannous chloride dihydrate in 100 ml. of 1 to 9 hydrochloric acid, prepared daily) were added in the order given, and mixed well after each addition. After 1 or 2 minutes the solution was extracted first with 10 ml. of ether (pretreated with one tenth its volume of equal amounts of the potassium thiocyanate and stannous chloride solutions), then with 5-ml. portions of ether until no additional color was served in the ether extract. The combined extracts were run into a 25-ml. volumetric flask (for very small quantities a 10-ml. volumetric flask was used) and diluted to volume with the treated ether. The solution was compared in a Duboseq colorimeter with a standard which was prepared simultaneously and in the same manner from the standard ammonium molybdate solution. Experiments conducted with various interfering ions which could normally be present in solutions of plant or soil samples confirmed the findings of Hoffman and Lundell that the presence of iron affects the molybdenum thiocyanate complex color. This is an enhancing effect which reaches a maximum when about 2 g. of iron are present, and additional iron has no further effect. Hoffman and Lundell recommend the addition of 10 mg. of ferric ion to every determination before reduction and extraction and this procedure was adopted in all cases where the iron was not readily present. Other ions, including  $\text{Ca}^{++}$  (200 mg.),  $\text{K}^{+}$  (60 mg.),  $\text{Na}^{+}$  (200 mg.),  $\text{Mg}^{++}$  (20 mg.),  $\text{Mn}^{++}$  (2 mg.),  $\text{Co}^{++}$  (0.2 mg.),  $\text{Zn}^{++}$  (0.2 mg.),  $\text{Cu}^{++}$  (0.2 mg.),  $\text{Zr}^{++++}$  (1 mg.),  $\text{Ti}^{+++}$  (1 mg.),  $\text{SO}_4^{--}$  (80 mg.),  $\text{Cr}_2\text{O}_7^{--}$  (0.7 mg.),  $\text{PO}_4^{--}$  (100 mg.),  $\text{B}_2\text{O}_7^{--}$  (2 mg.), and  $\text{SiO}_2^{--}$  (80 mg.) were added in the above amounts to both 500 and 50 micrograms of molyb-

denum and were found to give no interference in these proportions which are ordinarily encountered (23) in plant and soil samples.

Other experiments confirmed previous statements on the instability of the molybdenum thiocyanate color. Standards and unknowns were therefore prepared simultaneously and compared immediately. A calibration curve was prepared (24) by plotting the average reading—i.e., the arithmetic mean of 10 or more readings, with one cup set at some predetermined height—against the ratio of the solution concentrations in the two cups. Although a straight-line relationship exists, the indicated lack of conformity to Beer's law shows that the standards and unknowns should have a difference in concentration of not more than 25%.

The following procedure was finally adopted for analysis of the samples:

The material was first ignited at 450° C. and then carefully mixed. One gram of this ash was weighed out, and 10 ml. of (1 to 4) hydrochloric acid were added and heated. The solution was filtered through an ashless filter paper, washed several times with water, and the filter paper and residue were transferred to a platinum dish and ignited at 450° C. A few milliliters of water, several drops of sulfuric acid, and about 10 ml. of hydrofluoric acid were added, evaporated on a steam bath, and ignited. Water and hydrofluoric acid were added again and the evaporation was repeated. With plant materials there was often a residue of organic matter which was removed by again igniting the dish at 450° C. A few milliliters of water and 1 ml. of hydrochloric acid were added, warmed, and the solution was made up to 50 ml. With soils there was sometimes a residue which was filtered off. The hydrochloric acid concentration was adjusted, 10 mg. of iron were added if not already present, 3 ml. of potassium thiocyanate solution and 3 ml. of stannous chloride solution were added, the solution was extracted with several portions of ether, diluted to volume, and the color compared with a standard of approximately the same concentration prepared simultaneously. Using this procedure, duplicate analyses were made on the ten samples previously analyzed spectrographically. The results are given in column 4 of Table I.

The procedure used volatilizes the silicon as silicon tetrafluoride because it was found that molybdenum is often retained in the silica residue if a sodium carbonate fusion is used. The fusion also has the disadvantage of introducing some platinum into the solution, giving an interfering color which is especially serious when only a few micrograms of molybdenum are present. No molybdenum could be detected spectrographically in the residue from the hydrofluoric-sulfuric acid treatment nor was there any appreciable loss of molybdenum by this treatment, but the method is sensitive only to about 0.05 microgram.

To determine the accuracy and precision of the method a synthetic ash solution was prepared containing 100 mg. of  $\text{K}^{+}$  and  $\text{Ca}^{++}$ , 50 mg. of  $\text{Mg}^{++}$ , 10 mg. of  $\text{Na}^{+}$  and  $\text{Fe}^{+++}$ , 2 mg. of  $\text{Al}^{+++}$  and  $\text{Mn}^{++}$ , and 1 mg. of  $\text{Co}^{++}$ ,  $\text{Zn}^{++}$ ,  $\text{Cu}^{++}$ , and  $\text{Ni}^{++}$ , all as the chlorides, and 20 mg. of phosphorus as orthophosphoric acid. Known amounts of molybdenum were added and the analysis was carried out as previously described. Under these ideal conditions an accuracy of 5% and a probable error of 2.9% were secured. It is estimated that with plant or soil samples an accuracy of 15% could reasonably be expected.

The lower limit of detectability with this procedure is approximately 1 microgram, but the precision is rather poor with this amount in 10 ml. It is estimated that eight to ten determinations could be made in one day, although the speed and sensitivity might be increased by using photoelectric or microcolorimeters.

The advantages of this procedure are that no special apparatus is required and very small proportions of molybdenum may be determined. On the other hand, the residual solution is probably not suitable for other determinations and each determination requires relatively large amounts of sample (1 gram) which might not be available in all cases.

#### POLAROGRAPHIC METHOD

The polarograph used was a photographic recording Heyrovský instrument manufactured in Prague. The dropping electrode was made by sealing a 2.5-cm. length of Pyrex "marine

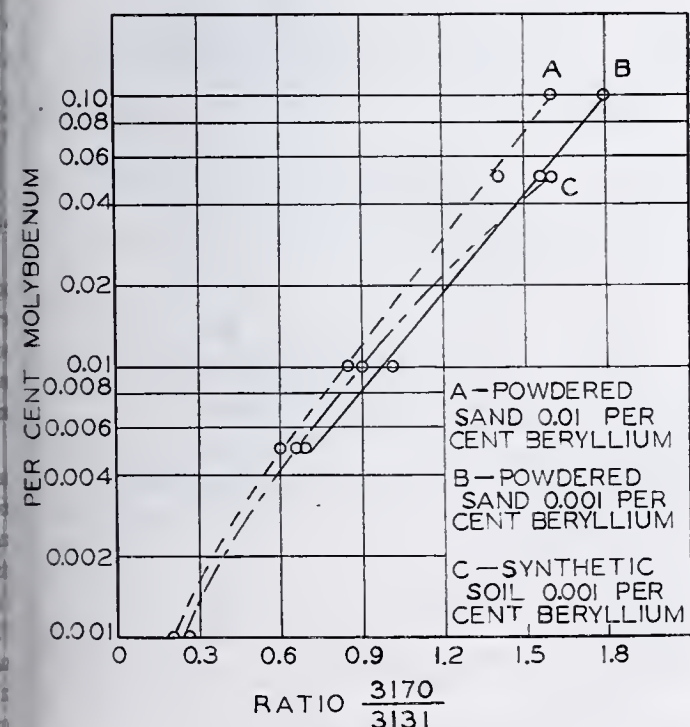


Figure 2. Plant Working Curve



barometer tubing" with an internal diameter of about 0.05 mm. into the bottom of a 50-ml. distilling flask with a 2.5-cm. length of Pyrex tubing. The distilling flask was connected to a manometer and leveling bulb containing mercury to maintain a suitable pressure and dropping rate (12).

Previous investigators, using fairly large amounts of molybdenum, have obtained satisfactory curves only in 18 *N* sulfuric acid (18), 33% phosphoric acid (8), and ammonium nitrate-nitric acid (22) solutions. Preliminary experiments were made with known amounts of molybdenum from the standardized ammonium molybdate solution and various regulating solutions by removing oxygen with nitrogen and analyzing polarographically with suitable galvanometer sensitivity. Regulating solutions of 0.4 to 70% phosphoric acid, 18 *N* sulfuric acid, sodium sulfate-sulfuric acid pH 1.5, sodium acetate-acetic acid pH 3.6, ammonium chloride-hydrochloric acid pH 1.1, 0.05 *M* potassium chloride, and ammonium nitrate-nitric acid pH 0.5 to 4.7 were used. Satisfactory curves were obtained with 20 to 35% phosphoric acid and various ammonium nitrate-nitric acid mixtures. In the latter case the step heights were markedly affected by changes in either hydrogen- or nitrate-ion concentration. The solution giving the most easily measured curves with sharper and better defined upper and lower "breaks" was one of pH approximately 1 and a nitrate concentration of 1.0 *N*. Uhl (22) recommended the use of lactic acid with this solution, but its addition gave unsatisfactory curves with maxima.

It was hoped that it would be possible to dissolve the plant or soil material in a suitable acid, adjust the pH and nitrate-ion concentration, and analyze the solution directly with the polarograph. A study of various other ions, in proportions such as might be expected in solutions of plants and soils, showed that potassium, calcium, sodium, and magnesium nitrates had no effect. While small amounts of potassium chloride (1 and 5 ml. of 1 *M* potassium chloride in 50 ml. of solution containing 5 microgram of molybdenum per ml.) did not have a great effect upon the character of the polarogram, larger amounts (10 and 25 ml.) affected the limiting current angle markedly. This may be due to the chloride ion, since equivalent amounts of potassium sulfate had no such effect. The presence of phosphoric acid reduced the step height and small amounts of iron gave a wave just preceding and close enough to that of the molybdenum to cause interference. The interference of one element with another may sometimes be eliminated by complex formation (10) and Uhl (22) stated that he could overcome the interference of iron by the addition of oxalate but not with citrate or fluoride. All were found unsatisfactory, as the first two either reduced the step height or introduced maxima and the fluoride eliminated the step altogether.

Attempts to remove the iron by precipitation with sodium hydroxide (13) or ammonium hydroxide (14) gave unsatisfactory results. The molybdenum was therefore separated by precipitation with alpha-benzoinoxime (9), dissolved, and the solution analyzed polarographically. The best conditions for precipitation of molybdenum with alpha-benzoinoxime are in solutions containing 5% nitric, hydrochloric, or sulfuric acid with an excess of reagent and below 10° C. Vanadium, chromium, tungsten, palladium, columbium, gold, or tantalum interferes but the last five seldom are encountered in plants and soils and the first two cause no trouble if they are reduced with a little sulfurous acid before the benzoinoxime is added.

Since no data were available on the precipitation of less than 50 micrograms of molybdenum in 200 ml. of solution, varying amounts of molybdenum were put in 50 ml. of solution containing 5% hydrochloric acid and analyzed. The solution was cooled below 10° C., and 2 ml. of a 2% alcoholic benzoinoxime solution and a few drops of bromine water were added. After standing 10 to 15 minutes with occasional stirring, the precipitate was filtered off, ignited at 450° C., the residue dissolved in 3 drops of *N* sodium hydroxide, 1 ml. 2 *N* nitric acid, and 2 ml. of 4 *N* ammonium nitrate added, the solution diluted to 10 ml., oxygen removed, and analyzed polarographically. The wave heights were evaluated by measuring, at the half-wave potential, the vertical distance between straight-line extensions of the principal slope lines through the centers of oscillation amplitudes before and after the current step. The results varied from 0.9%

loss with 100 micrograms, to 10% loss with 5 micrograms, a 30% loss with 1 microgram.

Known amounts of molybdenum were added to the same synthetic ash solution as used with the colorimetric method, a determined as outlined above. For comparison, solutions containing the same quantities of molybdenum, ammonium nitrate and nitric acid and 2 micrograms of iron (since the small quantities of iron carried down with the alpha-benzoinoxime caused a slight shift in the half-wave potential), were prepared and analyzed polarographically. A calibration curve for a drop rate 1.8 seconds and calculated to  $1/20$  galvanometer sensitivity showed a straight-line relationship between concentration and step height up to 5 micrograms of molybdenum per ml.

The soil and plant samples were prepared for precipitation as described for the colorimetric procedure. The hydrochloric acid concentration was adjusted to 5% and the precipitate made as above, adding a little sulfurous acid in the case of soil. With the soil samples containing a large proportion of iron, an alkaline solution of the residue after ignition was filtered through a small, sintered-glass funnel to remove the iron carried down with the precipitate. The results of the analysis of the plant and soil samples by this method are given in column 5 of Table I.

The accuracy of this procedure varies with the amount of molybdenum present and it should not be used for material containing less than 0.0005% molybdenum unless the sample is larger than 1 gram. It is estimated that 6 to 8 determinations could be made per day.

Because of the separation procedure, molybdenum is the only constituent which can be determined. Some copper is precipitated by the benzoinoxime, and gives a small step just preceding the molybdenum.

The corrected half-wave potential was determined to -0.42 volt, using a glass-jointed salt bridge as suggested by Kolthoff and Lingane (10).

The marked tendency of molybdenum to form heteropoly acids (2), the enhanced reducibility of these molybdenum heteropoly acids (3), and the fact that no step is obtained in hydrochloric acid solution but is in nitric, sulfuric, or phosphoric solutions make it seem likely that the step obtained in this study may be due to the reduction of a nitrate-molybdenum heteropoly acid.

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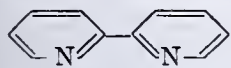
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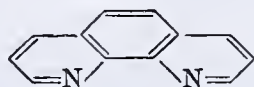
# A Selective Spot Reaction for Cadmium<sup>1</sup>

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In 1898 Blau (1) showed that  $\alpha, \alpha'$ -dipyridyl and  $\alpha, \alpha'$ -phenanthroline form intensely red, water-soluble, acid-resistant ferrous salts.



$\alpha, \alpha'$ -dipyridyl



$\alpha, \alpha'$ -phenanthroline

They owe their color to the complex ions  $\text{Fe}(\alpha, \alpha'\text{-dipyridyl})_3^{++}$  and  $\text{Fe}(\alpha, \alpha'\text{-phenanthroline})_3^{++}$ . These cations belong to the hexammine type, since six coordination positions of the iron atom are occupied by three molecules of base, each with two coordinating nitrogen atoms or rather  $\text{—N=}$  groups. In contrast to most ferrous salts, which readily undergo oxidation, these complex ions are remarkably stable, and are extraordinarily resistant against attack by even alkalis and alkali sulfides. The analytical application of these complexes came many years after their discovery.

In 1930, Hill (6), and then independently Feigl and his collaborators (3) in 1931, described the use of the dipyridyl compound for the detection and colorimetric determination of iron. The phenanthroline complex was used in the same way somewhat later. The numerous publications (5, 8, 9, 10, 12, 13, 15, 17, 18) testify to the value of these complexes for these purposes and also as redox indicators. Komarowski and Poluektoff (7) used the  $\alpha'$ -dipyridyl complex to detect molybdenum (after reduction with stannous chloride). Poluektoff and Nazarenko (11) described the microchemical detection of various anions through characteristic crystalline precipitates containing these complexes. Ferrari (4) utilized the formation of stable  $\text{Fe}(\alpha, \alpha'\text{-dipyridyl})_3^{++}$  ions to obviate the interference of iron in certain precipitation reactions of magnesium, beryllium, aluminum, and zirconium. The  $\alpha, \alpha'$ -dipyridyl acts as a masking agent in such cases.

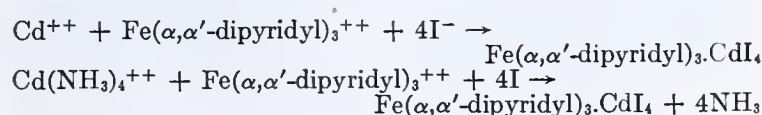
Some years ago, the senior author (2) showed that red precipitates result when considerable quantities of alkali iodides in dilute solutions of potassium-nickel cyanide are added to the solution of ferrous dipyridyl salts. The precipitates consist of  $\text{Fe}(\alpha, \alpha'\text{-dipyridyl})_3\text{I}_2$  and  $\text{Fe}(\alpha, \alpha'\text{-dipyridyl})_3\text{Ni}(\text{CN})_2$ , respectively. These observations suggested that solutions of these complex salts might function as general precipitants for voluminous anions. Exploratory trials in this laboratory have shown that such precipitates are formed by molybdic, tungstic,  $\text{H}_3\text{PO}_4$ ,  $\text{H}_2\text{MoO}_4$ ,  $\text{H}_3\text{PO}_4 \cdot 12\text{H}_2\text{O}$ , periodic, picric, picrolonic, and trinitrocarboxylic acids, and by the following complex anions:  $\text{GL}^{--}$ ,  $\text{BiI}_4^{--}$ ,  $\text{CdI}_4^{--}$ ,  $\text{Ni}(\text{CN})_4^{--}$ ,  $\text{Co}(\text{CN})_6^{--}$ ,  $\text{Zn}(\text{CN})_4^{--}$ ,  $\text{d}(\text{CN})_4^{--}$ , and  $\text{Hg}(\text{CNS})_4^{--}$ .

All the precipitates produced by the union of  $\text{Fe}(\alpha, \alpha'\text{-dipyridyl})_3^{++}$  or  $\text{Fe}(\alpha, \alpha'\text{-phenanthroline})_3^{++}$  with the foregoing compounds or ions are crystalline. Their color, which ranges from red, red violet, to blackish red, indicates that the red complex ions constitute the chromotropic constituent of the respective, slightly soluble salts.

The analytical utility of precipitating these complex anions with  $\text{Fe}(\alpha, \alpha'\text{-dipyridyl})_3^{++}$  and  $\text{Fe}(\alpha, \alpha'\text{-phenanthroline})_3^{++}$  has been studied in this laboratory. New methods of detection based on these precipitates have been developed, and furthermore new procedures for the quantitative determination of mercury, bismuth, nickel, zinc, cadmium, and phosphate have been worked out. However, satisfactory methods for detecting and determining these materials are already available, and in view of the relatively high price of  $\alpha, \alpha'$ -dipyridyl and  $\alpha, \alpha'$ -phenanthroline, it seems best at this time to limit the discussion to a single new application of these reagents, since it presents an actual advance and advantage.

The precipitation of red  $\text{Fe}(\alpha, \alpha'\text{-dipyridyl})_3\text{CdI}_4$  is the basis of a spot reaction that can reveal the presence of minute quantities of cadmium. If carried out in ammoniacal solution, this test succeeds even though copper and zinc are present. The simplicity of the procedure and the attainable identification limits and limiting proportions make it superior to all previous spot tests for this element. In addition, it appears probable that the precipitation of  $\text{Fe}(\alpha, \alpha'\text{-dipyridyl})_3\text{CdI}_4$  from ammoniacal solution will provide a simple method of quantitatively separating cadmium from considerable quantities of copper and zinc. This separation is extremely difficult when the methods available up to now are used.

The new method of detecting cadmium and separating it from other metals requires the maintenance of conditions at which there is no coprecipitation of red  $\text{Fe}(\alpha, \alpha'\text{-dipyridyl})_3\text{I}_2$  or other complex iodides. These conditions are easily secured by working in ammoniacal solution and by employing a saturated solution of  $\text{Fe}(\alpha, \alpha'\text{-dipyridyl})_3\text{I}_2$  that contains an excess of iodide ions. This reagent then contains all the ionic species requisite to the formation of  $\text{Fe}(\alpha, \alpha'\text{-dipyridyl})_3\text{CdI}_4$  in accord with the equations:



$\alpha, \alpha'$ -Phenanthroline may be used in place of the  $\alpha, \alpha'$ -dipyridyl.

## DETECTION OF CADMIUM IN THE PRESENCE OF OTHER METALS

**PRECIPITANT.**  $\alpha, \alpha'$ -Dipyridyl (0.25 gram) and ferrous sulfate heptahydrate (0.146 gram) are dissolved in 50 ml. of water. Ten grams of potassium iodide are added, the solution is shaken vigorously for 30 minutes, and the precipitate is filtered off. The filtrate is a saturated solution of  $\text{Fe}(\alpha, \alpha'\text{-dipyridyl})_3\text{I}_2$  containing an excess of potassium iodide. The reagent is stable. If it becomes cloudy after long standing, it can be restored to usefulness by filtering.

**PROCEDURE.** Thick filter paper (S. and S. 601 or Whatman 120) is used. A strip is laid across a small beaker or crucible. A drop of the weakly acidified, neutral, or ammoniacal test solution is placed on the paper. Before the drop is absorbed, the spot is treated with a drop of the precipitant. Reaction takes place at once, and after the liquid has soaked in, the cadmium-bearing precipitate is left as a red fleck or ring. The color is intense enough so that the precipitate is easily seen against the red stain left by the spreading of the reagent. A blank or comparison test is necessary only when very small amounts of cadmium are suspected.

The test solution and precipitant may also be brought together in a microcentrifuge tube. It is best to introduce the drop of the precipitant first, then a drop of the test solution, and to centrifuge immediately. For reasons that are not apparent, the sensitivity of the test is appreciably less if the drops are mixed before starting to centrifuge. When cadmium is present, a red precipitate collects in the constricted end of the tube.

Identification limit: 0.05 microgram of cadmium; limiting proportion: 1 to 1,000,000.

## DETECTION OF CADMIUM IN THE PRESENCE OF OTHER METALS

Metal ions that form slightly soluble or complex iodides interfere with the direct use of  $\text{Fe}(\alpha, \alpha'\text{-dipyridyl})_3\text{I}_2$  as precipitant for cadmium. The iodide of the reagent is thrown down by silver, thallium, and lead salts, and the precipitates are made red by adsorption of  $\text{Fe}(\alpha, \alpha'\text{-dipyridyl})_3^{++}$  ions. Cupric salts, in neutral or acidified solutions, liberate iodine with simultaneous precipitation of cuprous iodide, which forms colored addition complexes with  $\alpha, \alpha'$ -dipyridyl and  $\alpha, \alpha'$ -phenanthroline (16). Bismuth, mercuric, stannous, and stannic salts form soluble complex iodides, which likewise are deposited as red complexes

<sup>1</sup> Translated from the German manuscript by Ralph E. Oesper, University of Cincinnati.



containing  $\alpha, \alpha'$ -dipyridyl. (These latter precipitations are not nearly so complete as the corresponding cadmium reaction.)

None the less, the test for cadmium becomes almost specific if the test solution is treated beforehand with ammonia water. The interfering lead, mercuric, bismuth, stannous, and stannic ions are removed as hydrous oxides and the ammoniacal filtrate can then be tested with the reagent. The  $\text{Cd}(\text{NH}_3)_4^{++}$  ions in the filtrate react promptly, whereas any  $\text{Cu}(\text{NH}_3)_4^{++}$  and  $\text{Zn}(\text{NH}_3)_4^{++}$  ions are inactive. The only other interfering ions in the ammoniacal filtrate are  $\text{Ag}(\text{NH}_3)_2^+$  and thallium. If, therefore, cadmium is to be detected in the presence of these and other ions that form slightly soluble iodides the following procedure can be used:

Dilute hydrochloric acid is added to the test portion; any precipitate (silver, lead, thallous chloride) is filtered off. The filtrate is made ammoniacal, filtered if need be, and the clear solution tested with the reagent. All these operations can be carried out with one or two drops of the test solution in a microcentrifuge tube. The various precipitates are easily segregated by centrifuging.

The effectiveness of this test for cadmium was measured with the aid of ammoniacal solutions containing known quantities of  $\text{Cd}^{++}$  and  $\text{Cu}^{++}$ , and  $\text{Cd}^{++}$  and  $\text{Zn}^{++}$ . The findings were that in one drop it is possible to detect  $\left. \begin{array}{l} 0.08 \text{ microgram} \\ 0.1 \text{ microgram} \end{array} \right\}$  of cadmium in the presence of 5000 times this quantity of  $\left\{ \begin{array}{l} \text{copper} \\ \text{zinc} \end{array} \right.$ .

Far better limiting proportions are obtained if the detection of about 0.2 microgram of cadmium is all that is required.

#### QUANTITATIVE DETERMINATION OF CADMIUM

Although the precipitate conforms to the formula  $\text{Fe}(\alpha, \alpha'\text{-dipyridyl})_3\text{CdI}_4$ , the writers have not yet been able to use this material as the basis of a direct method for determining cadmium. When washed with water to remove the adhering reagent, the precipitate dissolves to some extent and also decomposes slightly. Consequently, low values are obtained. None the less, cadmium can be quantitatively separated from other metals, particularly copper and zinc, by precipitation from an ammoniacal solution by means of ferrous dipyridyl and iodide. After the  $\text{Fe}(\alpha, \alpha'\text{-dipyridyl})_3\text{CdI}_4$  has been isolated and decomposed, the cadmium can be determined by any of the accepted procedures. The precipitation by means of mercaptobenzothiazole (14) is recommended particularly.

The reagent solution used for the detection of cadmium is employed for its quantitative precipitation.

The precipitate is collected on paper or in a filtering crucible and washed with diluted precipitant, then dissolved in hot sulfuric acid (1 to 4). An excess of strong bromine water is added to the solution, which is then boiled for 10 minutes. The iron is thus oxidized and the iodide is converted to iodate. The cooled solution is made ammoniacal and then warmed. The hydrous iron oxide precipitate is filtered off. The cadmium is then precipitated by adding an ammoniacal solution of mercaptobenzothiazole to the clear filtrate. The precipitate is washed with dilute ammonia and brought to constant weight at  $110^\circ$  to  $120^\circ \text{C}$ . The factor to cadmium is 0.2330.

This procedure gave very satisfactory results with solutions that contained 100 mg. of cadmium along with 200 times this weight of copper, or 100 times this quantity of zinc. Lack of the reagent prevented trials on mixtures containing other proportions or other materials. It is hoped therefore that this promising procedure may be tried by other workers.

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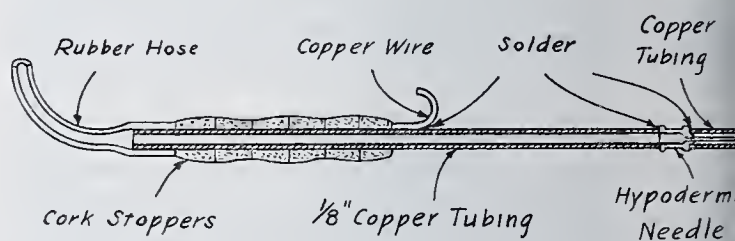
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## A Simple Microtorch

EDWARD N. DACUS

Beacon Research Laboratories, The Texas Company, Beacon, N. Y.

A HYPODERMIC needle (which can probably be obtained from a First Aid Room) makes an excellent nozzle for microflame. Details of a hand torch that has found many uses in this laboratory are shown in the accompanying diagram. The parts can be made with simple hand tools and soldered together to form a single unit. No special skill is required.



The beveled point is ground off the hypodermic needle and the shield is made to extend about 4.7 to 6 mm. (0.188 to 0.236 inch) beyond its tip. The only function of the shield is to protect the flame from drafts. Air holes in the base of the shield are neither necessary nor desirable. A nonreducing flame is obtained without them, and they tend to decrease the stability of the flame, which otherwise is extremely difficult to extinguish by drafts or sudden movements through the air.

The convenient hand grip shown in the diagram can be provided, as an additional refinement, by boring several cork stoppers and slipping them over the barrel of the torch with their large and small ends alternately facing. They should be shellacked in place and have their edges sanded smooth.

A Becton and Dickinson No. 25 needle, which has an inside bore of the order of 0.25 mm. (0.01 inch), gives a nonreducing "pin flame" that is hot enough for soldering electrical connections and small parts. A still hotter flame, for work with quartz fibers and the like, can be made by using a slightly larger (No. 18) needle and feeding oxygen into the gas through a T placed well back in the rubber tubing. In such case, the gas should be turned on and lighted before admitting oxygen to the line, and the oxygen should be turned off before turning off the gas.

A gas flame about 1 mm. in diameter and from 1 to about 30 mm. in length can be obtained with a No. 25 needle. With a No. 18 needle and a somewhat shorter overhang of the shield a gas-oxygen flame about 3 mm. in length is obtained. When no further tests have been made, it is obvious that the size of the flame can, within limits, be varied to suit by proper choice of needle size.



# NEW EQUIPMENT

## Carbon Determinator

The Harry W. Dietert Co., Detroit, Mich., announces an improved 2-minute carbon determinator for rapid and accurate total carbon determinations of all metals.

The gas pressure in both the measuring buret and carbon dioxide absorption vessel is automatically brought to the same atmospheric pressure, thus eliminating the possibility of inaccurate reading due to the difference in gas pressure at the start and the end of the test. The gas pressure in the measuring buret is precisely brought to atmospheric pressure at the time the reading is taken. The buret is connected to an absorption vessel constructed in the form of a U-tube, and the liquid level is brought to an exact fixed hairline marker on a capillary tube stem of this vessel. This marker is set at atmos-

pheric pressure level and eliminates the error usually obtained in leveling by eye between the aspirator bottle and buret stem. The sample is burned in the combustion tube in a partial vacuum which aids in complete combustion.

## Refractometer

A continuous-flow, pressure-type refractometer has been developed by the Shell Development Co., Emeryville, Calif., for indicating the purity of products flowing through a processing pipe line.



The instrument has been manufactured and marketed by the Precision Scientific Co., and is used by Shell in the production of butadiene. It utilizes the measurement of the physical factor refractive index as an indication of purity.



Leeds & Northrup Portable Universal pH Indicator for Measuring Grab Samples in Laboratory or Plant

## Electric Molecule Sorter

Production of high-octane gasoline, butadiene, or styrene involves precise critical processes. Westinghouse Research Laboratory, East Pittsburgh, Pa., has developed a control mechanism that thoroughly checks operation in a matter of minutes and requires only one or two technicians.

The mass spectrometer determines both qualitatively and quantitatively the constituents of a gas. It uses only a small quantity of the gas and ionizes it by impact of electrons from a hot filament in an evacuated tube. The stream of charged molecules is drawn along the tube into a strong magnetic field, where it is bent into an arc-shaped path. The heavier the ion, the larger the radius of curvature of its path. As a result, different molecules emerge from the field at various locations, but all of the same kind leave at one particular spot. Different charged molecules are collected successively at an exit slit into a current that can be amplified and measured.

Built for use in refineries, chemical plants, and similar locations the mass spectrometer is a self-contained tool. No power supply other than 110 volts, 60 cycles, is required. The instrument is extremely sensitive and can measure ion beams as small as 0.000000001 micro-ampere. It can readily detect, for example, the presence of one part of oxygen in 10,000 parts of nitrogen.

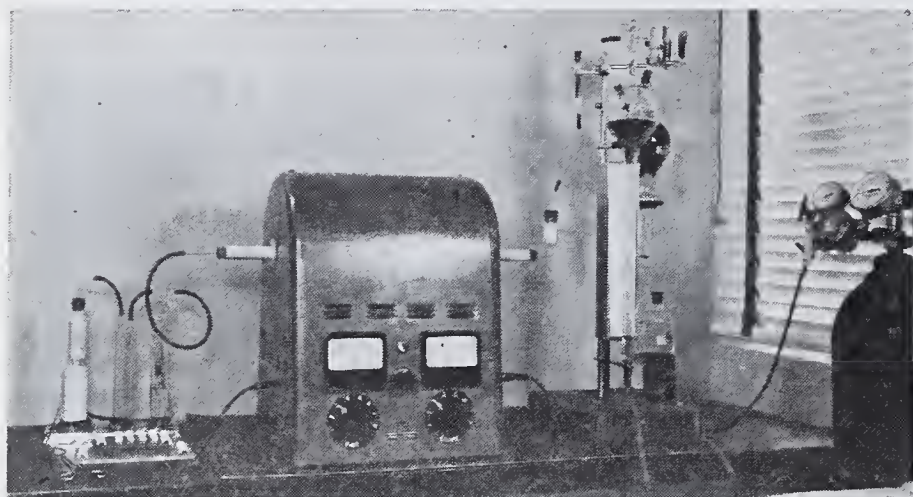
## Resiliometer

To measure resiliency is the function of an instrument known as the Resiliometer, manufactured by the Precision Scientific Co., 1736 North Springfield Ave., Chicago, Ill. Resiliency of rubber and extensible plastic compounds is indicated by the rebound of a weighted plunger dropped on the test specimen from a predetermined height.



## Laboratory Combustion Tube Furnace

In announcing the new combustion tube furnace for the laboratory, Lindberg Engineering Co., Chicago, Ill., calls attention to its combined utility and modern streamlined design.



Laboratory Combustion Tube Furnace

The CF-1, a single-tube furnace, is designed for carbon and sulfur determinations as well as gravimetric determination of carbon and alloy steels. Temperatures obtainable for continuous operation are up to 2500° F. while for occasional operations temperatures up to 2650° F. are permissible. High-temperature Globar heating elements are easily installed or removed without total dismantling and assembling of the unit.

All necessary control is conveniently located on face of furnace. Temperature regulation is provided by coarse and fine adjustment knobs connected to a built-in variable-voltage transformer. An ammeter shows the proper and safe current required for the Globar heating elements, while temperature is indicated by a pyrometer connected to a platinum thermocouple. A knob for controlling the flow of oxygen operates a needle valve connected to rubber tubing adapter located at both ends of the housing for piping to the oxygen supply.

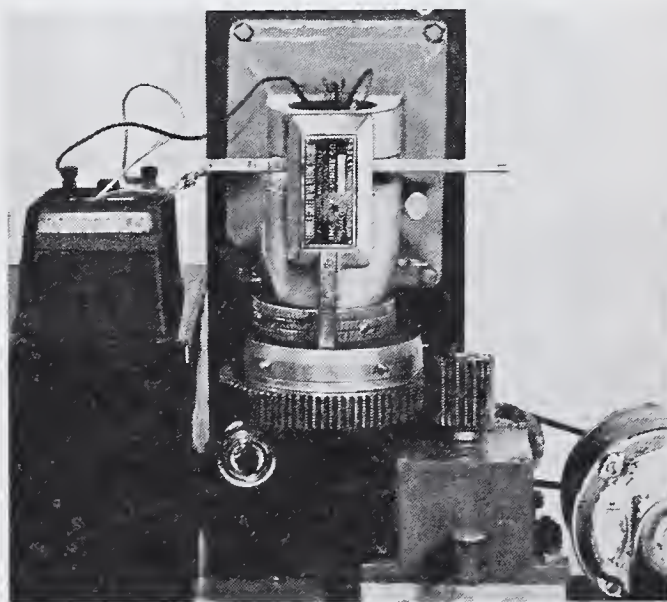
## New Machines for Paint Testing

Two new machines for controlled testing, measurement, and evaluation of the flexibility, scratch hardness, and adhesion of paints and other coatings, have been announced by Kam N. Kathju, technical director of The Arco Co., Cleveland, Ohio. The machines were developed to provide for accurate measurement of the basic characteristics and progressive deterioration of surface films.

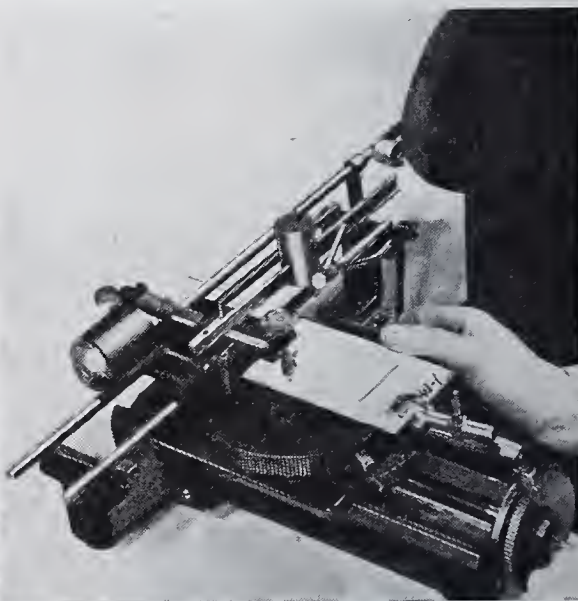
Tests and measurements of flexibility are made on the Elongauge, which has been developed around a conventional Erichsen sheet metal testing machine, adapted to provide complete automatic controls and accurate measurements for cycle testing. The machine is mounted with its observation well in a vertical position, so that water

completed by the presence of a copper needle causes a current to flow. This deflects the galvanometer which signals the end point of the test. The thrust is measured in 0.01 mm. and is converted to per cent elongation by a fixed table.

Scratch hardness and adhesion are measured by the Microknife, a diamond-point cutting tool applied to the surface being tested by a lever arrangement carrying beam and weight. The load, measured in grams, is applied to the point which moves across the surface at constant speed and cuts repeatedly in a fixed position until the surface is revealed. The load on the beam and the number of strokes required to wear through the film are the measure of scratch hardness.



Elongauge



Microknife

from a hypodermic syringe is in contact with the paint film being tested. Two wires, one attached to the test panel and the other to the hypodermic needle, are connected to a galvanometer. The machine is operated by a constant-speed drive geared to thrust a  $\frac{3}{16}$ -inch spindle against the back of the test panel which is clamped between two anvils. The paint film being tested acts as an insulator. When it fails, the water contacts the steel test panel and the bimetal condition

Used in connection with a movable platform which can be adjusted laterally by a precise screw thread and notched wheel, the Microknife becomes an accurate adhesion measuring machine. A standard stroke is applied at progressively smaller spacings until it is sufficient to displace the coating in the area between cuts. It is possible to measure the relative adhesion of a coating to base metals and subcoatings, as well as to record changes in adhesion caused by aging of the paint.



## FAC Color Standards for Commercial Fats

Governmental agencies have established trading grades for tallow and greases based upon FAC colors. Considerable confusion has been caused, since the FAC color set as originally devised was not based on colors progressively darker as the number of the tubes increased.

American Oil Chemists' Society methods define the set as follows:

This set consists of 26 color standards, numbered with odd numbers from 1 to 45 and divided into 5 series. Numbers 1 to 9, inclusive, for light colored fats; numbers 11, 11A, 11B, 11C for very yellow fats; numbers 13 to 19, inclusive, for comparatively dark fats of a reddish cast; numbers 21 to 29, inclusive, for fats with a greenish cast; numbers 31 to 45, inclusive, for very dark fats.

The tube numbering was done arbitrarily to identify the tubes on the basis of hue, so that in many cases a tube of higher number is not darker but distinctly lighter than tubes of lower number. Obviously, since all the FAC color numbers were not included when the governmental grades were established, there is much misunderstanding as to where fats of various FAC readings should be classified.

The Fat Analysis Committee, a joint committee of the American Oil Chemists' Society and the AMERICAN CHEMICAL SOCIETY, and originator of the present FAC color standards, prescribes the following interpretation of the relationship of the FAC color standards:

FAC Tube No.	Tubes Listed Below Are Equal to or Lighter than Corresponding Tube in the Left-Hand Column
1	1
3	1, 3
5	1, 3, 5
7	1, 3, 5, 7
9	1, 3, 5, 7, 9
11	1, 3, 5, 7, 9, 11
11A	1, 3, 5, 7, 9, 11, 13, 11A
11B	1, 3, 5, 7, 9, 11, 13, 15, 11A, 11B
11C	1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 11A, 11B, 11C
13	1, 3, 5, 7, 9, 11, 13, 11A
15	1, 3, 5, 7, 9, 11, 13, 15, 11A, 11B
17	1, 3, 5, 7, 9, 11, 13, 15, 17, 11A, 11B
19	1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 11A, 11B, 11C
21	1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 31, 33, 11A, 11B, 11C
23	1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 31, 33, 35, 11A, 11B, 11C
25	1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 31, 33, 35, 37, 11A, 11B, 11C
27	1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 31, 33, 35, 37, 39, 11A, 11B, 11C
29	1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 11A, 11B, 11C
31	1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 31, 11A, 11B, 11C
33	1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 31, 33, 11A, 11B, 11C
35	1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 31, 33, 35, 11A, 11B, 11C
37	1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 31, 33, 35, 37, 11A, 11B, 11C
39	1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 31, 33, 35, 37, 39, 11A, 11B, 11C
41	1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 31, 33, 35, 37, 39, 41, 11A, 11B, 11C
43	1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 11A, 11B, 11C
45	1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 11A, 11B, 11C

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## Laboratory Reports to WPB

Laboratories carrying on essential research and production control activities, and which have been assigned serial numbers under Order P-43, were not required to file reports on Form WPB-167 on January 1, 1943, and will not have to file quarterly reports, although WPB may require reports at any time deemed desirable. The change in requirements is given in Direction I to Order P-43.

## Book Reviews

**A Short Course in Quantitative Analysis.** Hobart H. Willard, N. Howell Furman, and John F. Flagg. IX + 253 pp., 28 figures, 14 × 22 cm. D. Van Nostrand Co., New York, N. Y., 1943. Price, \$2.50.

**Advanced Quantitative Analysis.** Hobart H. Willard and Harvey Diehl. XI + 457 pp., 40 figures, 14 × 22 cm. D. Van Nostrand Co., New York, N. Y., 1943. Price \$4.75.

These two books are designed for two distinct purposes. The first has been written for the one-semester course being offered in many colleges to meet the needs of students majoring in fields other than chemistry, who should nevertheless have a background in quantitative analysis for more specialized study in the field of their special interest. The second is meant to serve as a textbook for advanced courses given to those taking graduate training in chemistry. It is called a companion volume to "Elementary Quantitative Analysis" by Willard and Furman and is written on the assumption that the student is already familiar with the fundamental theory and common operations of quantitative analysis as found in that volume. There are frequent references to the earlier book. Both books contain many footnotes referring the student to the literature of analytical chemistry. Both are essentially texts in inorganic quantitative analysis, although the word "inorganic" does not appear in either title.

The Short Course contains three chapters (44 pages) of introductory matter, eight chapters (118 pages) on volumetric analysis, and three chapters (57 pages) on gravimetric analysis. Volumetric analysis precedes gravimetric, but the order can be readily reversed if desired. A number of problems are given at the end of each chapter.

The introductory chapters contain a minimum of fundamental theory; notes on common operations and common equipment; necessary information about the construction, use, and care of the analytical balance; and a clear and concise discussion of scientific measurements.

The section on volumetric analysis opens with a short discussion of general principles. There are chapters on acidimetry and alkalimetry, volumetric precipitations, and oxidation-reduction. Under the last heading are found procedures involving the use of ceric sulfate as well as permanganate, dichromate, and iodine. In addition to preparation and standardization of reagents, the exercises include determination of Na<sub>2</sub>O in Na<sub>2</sub>CO<sub>3</sub>, replaceable hydrogen, Kjeldahl nitrogen, chloride, iron, calcium, available oxygen in pyrolusite, available chlorine in bleaching powder, copper, and arsenic. In some cases more than one procedure is given.

The student is introduced to gravimetric analysis by a discussion of the guiding principles of analytical precipitations, washing of precipitates, drying and ignition of precipitates, and calculations. The exercises include determination of chloride, sulfate, magnesium, and phosphate and finally the "proximate analysis" of limestone.

The exercises are well chosen and are ample in number to give the instructor freedom of selection. The principle of each method is clearly outlined before the actual details of the procedure are given. In many cases, sources of error, interfering substances, and other applications are discussed briefly. In the effort to limit the scope of the book and design it for those not majoring in chemistry there is perhaps a little oversimplification. In general, however, the authors have produced a textbook which should appeal to teachers of the short course.

Advanced Quantitative Analysis may, like the first, be conveniently broken into three sections. Quoting from the preface:

The first third of the book is devoted to a discussion of the general methods of chemical analysis; the topics are taken up in the order in which they are met in the course of an analysis, the problems of sampling, the methods of decomposing and dissolving materials, the methods of concentrating small amounts of materials, the various methods of separating the elements, and so on through to the methods of reporting the results. The second third is devoted to the analysis of iron ore, steel, and silicate rock, and has sections devoted to the analytical chemistry of each of the elements encountered. Directions



for laboratory work accompany this portion. The last third is a discussion of the analytical chemistry of each of the elements not studied earlier; the common and most of the rarer elements are treated. The order in which the elements are discussed is that of the periodic table, and sufficient information is given to supply the student with the basic information needed for solving the problems involved in the analysis of complex inorganic mixtures.

The first section also contains information on use and care of platinum, use of perchloric acid, commoner organic precipitants, and factors to be considered in evaluating a proposed method.

In the second third of the book, one chapter (III) is devoted to iron ore, iron, and steel. In addition to iron itself, the determination of the minor constituents, Al, Ti, Mn, Si, S, P, and C, is discussed. The analytical chemistry of these elements is well presented, and in most cases alternate procedures for their determination are given. In the analysis of alloy steels the discussion is limited to four alloying elements, W, Mo, Cr, and V, and their chemistry as it relates to analysis is stressed. The last chapter refers to determination of the alkali metals and to the prior decomposition of insoluble silicates.

The third part contains a discussion of the elements not already considered, arranged according to the periodic table. After a summary of the characteristics of the group, each element is treated separately. The discussion includes notes on methods of separation and gravimetric, volumetric, and colorimetric methods of determination. This chapter contains two excellent tables, the first summarizing the principal methods for the separation and gravimetric determination of the elements, the second the principal reactions useful for volumetric determinations. The text concludes with some notes on the determination of atomic weights.

The book is somewhat unusual in its general treatment of the subject. It contains very little of physico-chemical theory and stresses instead factual chemistry pertinent to the analysis of the elements. It is written as a textbook of chemical rather than physico-chemical or instrumental methods, although a few topics in the latter category (electrodeposition, potentiometric titrations, and colorimetry) are included. With the exception of the part on iron and steel and silicates, the choice of laboratory work seems to be left largely to the instructor, which is perhaps as it should be in a book designed for graduate courses. The student is expected to obtain from the text a knowledge of the important separations of a given element from interfering elements, of the forms in which the element may be determined gravimetrically, and the equations for the reactions used in the volumetric determination. He applies his knowledge in drawing up his own scheme for the quantitative analysis of an unknown after first making a careful qualitative analysis. This preview of, and practice in, the approach he will have to use if he undertakes industrial analysis is to be commended.

Although written for use as a text, the book should also be a useful addition to the reference shelf of the analytical chemist.

R. P. CHAPMAN

## A.S.T.M. Standards on Paper and Paper Products

This compact booklet includes all the standards of the American Society for Testing Materials on paper and paper products. Three specifications are used for electrical insulation, one is for waterproof paper for curing concrete, and two pertain to quicklime for paper manufacture. Most of the book is devoted to the 30 test methods which include procedures for alkali staining, ash content, casein, compression testing, folding endurance, grease resistance, moisture, opacity, resistance to passage of air, surface wettability, tearing resistance, thickness, and basis weight. In much of its work Committee D-6 has cooperated closely with T.A.P.P.I.

Copies in heavy paper cover can be obtained from A.S.T.M. Headquarters, 260 South Broad St., Philadelphia 2, Pa., at \$1.35 each.

## Volatile Matter in Coal

Following a study requested by a committee of the American Society for Testing Materials, the Bureau of Mines has published a report dealing with errors of analysis in the determination of volatile matter in anthracite, low-temperature coke, and subbituminous coal. The report, prepared by W. A. Selvig, states that the American Society for Testing Materials permissible differences between duplicate determinations of volatile matter by the same laboratory on the same sample of fuel should be revised to permit

0.3% for anthracite, 0.5% for low-temperature coke, and 0.7% for subbituminous coal. It would be advantageous if all laboratories adopted for all fuels the 10-cc. platinum crucibles with capsule-type covers and employed electrically heated vertical tube furnaces such as are used in the laboratories of the Bureau of Mines.

A copy of Report of Investigation 3739, "Precision of the Volatile Matter Determination for Anthracite, Low-Temperature Coke, and Subbituminous Coal", may be obtained from the Bureau of Mines, Department of the Interior, Washington 25, D. C.

## Spectrographic Steel Standards

The National Bureau of Standards is prepared to furnish a number of steels in rod form suitable for spectrographic standards. The standards now available were selected for checking important representative points of composition among several types of carbon and low-alloy steels. The values of concentrations are sufficiently well distributed in most cases to provide analytical curves for Mn, Cu, Ni, Cr, V, and Mo.

The standards are issued in two sizes: (1) cylindrical rod  $\frac{7}{32}$  inch in diameter, 4 inches long (approximately 22 grams), and (2) cylindrical rod 0.5 inch in diameter, 2 inches long (approximately 10 grams). The standards are numbered in consecutive order in two series, the first beginning with 401 for the  $\frac{7}{32}$  inch rods and the second beginning with 801 for the 0.5-inch rods. The standards may be ordered by number from the National Bureau of Standards, Washington, D. C., at \$3.00 per sample, in either series, irrespective of the number of samples ordered.

## Aniline Points of Hydrocarbons

The Bureau of Mines has published a comprehensive report on aniline points of hydrocarbons, one of the yardsticks for determining the quality of gasoline, Diesel oil, lubricants, and other products. Compiled with the cooperation of the University of Wyoming, the report summarizes literature on aniline points of pure hydrocarbons with 82 references to the original publications and presents a method for correlation of aniline points, and a selection of the best value for the aniline point for more than 400 hydrocarbons.

A copy of Report of Investigation 3721, "Aniline Points of Hydrocarbons", by John S. Ball may be obtained from the Bureau of Mines, Department of the Interior, Washington 25, D. C.

## Explosibility of Metal Powders

The explosive and inflammable characteristics of some of the metal powders now widely used in American wartime plants have been determined by Bureau of Mines research workers in a move to assist industry in minimizing or preventing accidents and to assure the uninterrupted production of vital materials. Zirconium, magnesium, and magnesium alloys, aluminum, and titanium powders are described, as "the most inflammable" of the 53 metal powder samples from 14 different metals and two alloys studied.

A copy of Report of Investigation 3722, "The Inflammability and Explosibility of Metal Powders", by Irving Hartmann, John Nagel, and Hylton R. Brown, may be obtained by writing the Bureau of Mines, Department of the Interior, Washington 25, D. C.

**Textbook of Quantitative Inorganic Analysis.** I. M. Kolthoff and E. B. Sandell. Revised edition. 794 pages. Macmillan Co., New York, N. Y., 1943. Price, \$4.50.

The subject matter and organization of the book are essentially the same as in the 1936 edition, but changes and additions have been made to bring it up to date. Fuller treatment has been given organic reagents, spectrophotometry, and errors, and a discussion of amperometric titrations has been introduced.

**Directory of Biological Laboratories.** 2nd edition, revised. 900 pages. Burns Compiling & Research Organization, 200 Railway Exchange Bldg., Chicago, Ill., 1943. Price, \$3.00.

This completely revised edition identifies and lists approximately 800 laboratories concerned with biological, bacteriological, or biochemical investigations, including research and commercial consulting laboratories and those related to manufacturing processes.



# Systematic Polarographic Metal Analysis

## Analysis of the Copper Group with the Aid of Electrolytic Separations

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best supporting electrolyte for the simultaneous polarographic determination of copper, bismuth, lead, and cadmium contains 0.4 M sodium tartrate, 0.1 M sodium hydrogen tartrate, and not more than 0.005% gelatin as a maximum suppressor. Concentrations of gelatin larger than 0.03% obliterate the bismuth wave. When one member of the nobler members of the group predominates and thus interferes with the polarographic determination of the others, the interfering constituents may conveniently be removed by electrol-

ONE of the most attractive features of the polarographic method as applied to metal analysis is that relatively few physical separations are necessary, and interferences can be eliminated in many instances simply by proper choice of supporting electrolyte (2, 4). However, in a systematic scheme that provides for the detection and determination of a dozen or more metallic elements, a few preliminary group separations are a practical necessity; otherwise interferences become too numerous to be circumvented simply by changing supporting electrolytes. It is fortunate that the hydrogen sulfide and ammonium sulfide groups of the classical qualitative analytical scheme happen to be at least as, or more, amenable to subsequent polarographic analysis than any other two combinations of the metals concerned, and separation into these groups will be employed in the systematic polarographic scheme that is being developed in this laboratory. Furthermore, in the analysis of the hydrogen sulfide group it is planned to follow classical procedure through one more step and separate the sulfides by extraction with strongly alkaline sulfide solution into a copper group (copper, bismuth, lead, and cadmium) and a tin group (tin, antimony, and mercury), because this simple separation greatly facilitates subsequent polarographic determinations and creates a maximal number of opportunities for simultaneous determinations.

The general polarographic characteristics of the metals of the hydrogen sulfide group in various supporting electrolytes are discussed in the first paper of this series (4), which also described a simplified method of computing concentrations by the use of previously determined diffusion current constants. The present paper presents detailed information concerning optimum conditions for analysis of the copper group, and describes an electrolytic procedure for separating interfering large amounts of the nobler members of the group from small amounts of the less noble members prior to the determination of the latter.

### OPTIMUM CONDITIONS FOR POLAROGRAPHIC ANALYSIS OF THE COPPER GROUP

The general characteristics of the waves of copper, bismuth, lead, and cadmium in a number of supporting electrolytes, and the technique of the polarographic measurements, have been discussed (4). When all members of the group are present an

analysis with a mercury cathode at a carefully controlled potential, and the minor baser metals are then determinable in the residual solutions. A mercury cathode is uniquely advantageous for electrolytic separations because the optimum values of the cathode potential may be deduced reliably from the known polarographic characteristics of the metals to be separated. An apparatus and technique for performing such separations are described in detail, and the efficiency of the method is illustrated by typical examples.

acidic tartrate solution is the only supporting electrolyte of those investigated in which their half-wave potentials differ sufficiently to permit their simultaneous determination. The use of a tartrate supporting electrolyte for the simultaneous determination of copper, bismuth, lead, and cadmium was recommended originally by Suchy (7), who published polarograms showing well-separated waves of these metals in strongly alkaline as well as acidic tartrate media. The writer has never been able to duplicate the satisfactory polarograms from alkaline tartrate media reported by Suchy. According to the author's experience (4) copper and bismuth do not produce satisfactory waves in alkaline tartrate solutions although lead and cadmium do, and an acidic tartrate medium must be employed for the simultaneous determination of all members of this group. When all four metals are present the optimum pH range of the tartrate supporting electrolyte is between about 4 and 4.5.

At a pH equal to or smaller than that of a pure sodium hydrogen tartrate solution (ca. 3.6) the copper and bismuth waves tend to coalesce, and in solutions of pH much greater than about

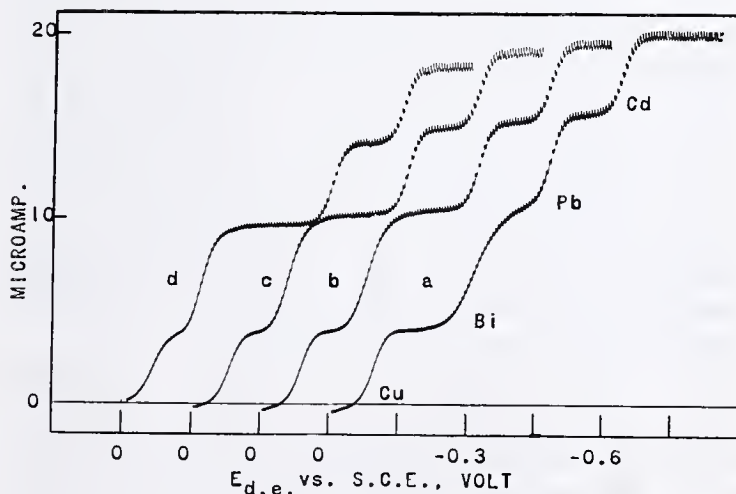


Figure 1. Polarograms of Copper Group Metals in Tartrate Media of Various pH

Mixture of ca. 0.8 millimolar copper, bismuth, lead, and cadmium, in tartrate media of various pH values. Total concentration of sodium tartrate and sodium hydrogen tartrate was 0.5 M, and the pH values were (a) 5.5, (b) 4.5, (c) 4.1, and (d) 3.6.



5, the bismuth wave is not well developed when gelatin is used as a maximum suppressor. These facts are demonstrated by the series of polarograms in Figure 1.

Curve *a* was obtained with a solution that contained approximately 0.8 millimolar copper, bismuth, lead, and cadmium, in 0.5 *M* sodium tartrate containing 0.02 *M* sodium hydrogen tartrate, 0.1 *M* sodium chloride, and 0.005% gelatin as a maximum suppressor (pH 5.5). Curves *b*, *c*, and *d* were recorded after successive additions of a standard nitric acid solution, so that solution *b* contained 0.4 *M* tartrate ion and 0.1 *M* hydrogen tartrate ion (pH about 4.5), solution *c* contained 0.3 *M* tartrate ion and 0.2 *M* hydrogen tartrate ion (pH about 4.1), and solution *d* corresponded to 0.5 *M* sodium hydrogen tartrate (pH about 3.6). The decrease in the wave heights from *a* to *d* resulted from the dilution produced by the addition of the nitric acid, and not from a change in the diffusion current constants.

The half-wave potentials and other characteristics of the copper, lead, and cadmium waves are not altered very much by changes in pH between about 3.6 and 6 in these tartrate media, but the half-wave potential of bismuth undergoes a pronounced shift to a more positive value with decreasing pH. The half-wave potential of bismuth decreases from  $-0.31$  volt at a pH of about 5.5 (curve *a*) to  $-0.17$  volt at a pH of about 3.6 (curve *d*), whereas the half-wave potentials of the other metals decrease by only about 0.03 volt. Consequently, with decreasing pH below about 5 the bismuth and lead waves become better separated but the copper and bismuth waves approach coincidence. The best supporting electrolyte for the simultaneous determination of all four metals when they are present at about equal concentrations is one containing about 0.4 *M* tartrate ion and 0.1 *M* hydrogen tartrate ion, corresponding to curve *b* in Figure 1. In this supporting electrolyte, and in the presence of 0.005% gelatin (see below), the half-wave potentials of copper, bismuth, lead, and cadmium are, respectively,  $-0.09$ ,  $-0.23$ ,  $-0.48$ , and  $-0.64$  volt *vs.* the saturated calomel electrode, and their diffusion current constants (*I*) are 2.37, 3.12, 2.37, and 2.34 microamperes per millimole per liter at 25° for  $m^{2/3}t^{1/6} = 1$ . These latter values may conveniently be used, instead of individual calibrations with known solutions in every case, to compute concentrations from observed diffusion currents (*I*).

For the determination of a small amount of copper in the presence of a very large amount of bismuth, a somewhat larger ratio of tartrate ion to hydrogen tartrate ion than 4 to 1 is preferable (curve *a*, Figure 1).

The concentration of gelatin used as a maximum suppressor has a very marked effect on the properties of the bismuth wave, as shown in Figure 2. Without gelatin (curve *a*) the bismuth wave shows a sharp maximum, although the other waves are fairly well developed. With 0.005% gelatin (curve *b*) all four waves are well defined, but with 0.01% gelatin the bismuth wave becomes flattened and a definite diffusion current plateau is not observed before the reduction of lead begins (curve *c*). With 0.03% gelatin (curve *d*) the bismuth wave is obliterated completely, and the diffusion currents of lead and cadmium are greatly suppressed. The suppressive effect of gelatin on the waves of other metals has been noted before (2). When bismuth is present the concentration of gelatin should not exceed 0.005%, and even if bismuth is absent it should not be larger than 0.01%. In spite of this one undesirable quality, gelatin remains one of the best and most generally applicable maximum suppressors when it is used with discretion.

Moderate concentrations (up to about 0.2 *M*) of sodium nitrate or sodium chloride, which may accumulate in practical analyses, have no appreciable influence on the properties of any of the waves when the total concentration of sodium tartrate and sodium hydrogen tartrate is 0.5 *M*. More than small amounts of potassium salts cannot be present in the acidic tartrate solutions because of the limited solubility (ca. 0.03 *M* in pure water) of potassium hydrogen tartrate.

When a large—e.g., 0.01 *M*—concentration of lead is added

to an acidic tartrate solution it partly precipitates, appearing as lead hydrogen tartrate. From measurements of the diffusion current of the lead in equilibrated solutions the solubility of the precipitate was found to be  $1.77 \times 10^{-3}$  *M* at 25° C. in a solution containing 0.4 *M* sodium tartrate and 0.1 *M* sodium hydrogen tartrate. However, the precipitate readily forms supersaturated solutions which are quite stable, and it is possible to work with concentrations of lead up to about 5 millimolar if the polarographic measurements are made within about an hour after the solutions are composited. For example, in one instance a solution containing 9.1 millimolar lead ion was stable for over an hour and a half at 25° C. with nitrogen bubbling through it, but when precipitation was induced by seeding and scratching the glass wall of the cell it proceeded rapidly, and solubility equilibrium was attained after about 10 minutes.

#### COMBINATION OF ELECTROLYTIC SEPARATIONS WITH POLAROGRAPHIC ANALYSES

It is evident that a single polarogram from an acidic tartrate supporting electrolyte will suffice for the simultaneous determination of all members of the copper group when they are present at very nearly equal concentrations, but in actual practice such an ideal situation cannot be expected, and a generally applicable systematic scheme of analysis must provide for those instances where one or more of the group predominate and thus interferes with the determination of the others. The predominance of a less noble metal, such as lead, will not interfere with the determination of a more noble metal, such as copper, because the wave of the latter is well in advance of that of the former, and traces of copper can be determined in the presence of an excess of bismuth, lead, or cadmium. In general, when the concentrations increase in the order copper, bismuth, lead, cadmium, a complete analysis of the group can be achieved by simply recording four polarograms of the same solution with successively smaller galvanometer sensitivities, so as to magnify in turn the wave of each constituent to a value which will allow accurate measurement. In the opposite case, when the concentrations decrease in the order copper, bismuth, lead, cadmium, the larger waves of the nobler metals prevent accurate measurement, or in extreme cases even the detection of the smaller waves of the baser metals, and it is then necessary to eliminate the waves of the nobler metals.

There are three possible methods that might be used to eliminate the interference of the intrinsically nobler metals: (1) physical separation by precipitation, distillation, extraction,

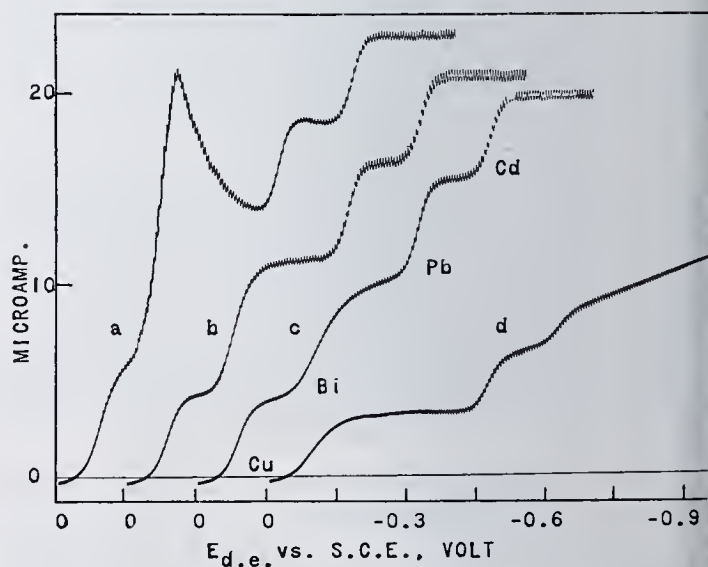


Figure 2. Influence of Gelatin

Mixture of ca. 0.8 millimolar copper, bismuth, lead, and cadmium, in acidic tartrate medium of pH 4.5. Concentrations of gelatin were (a) 0, (b) 0.005, (c) 0.01, (d) 0.03%.



or electrodeposition; (2) hanging the supporting electrolyte so that the order of the waves is reversed (usually as a result of complex ion formation); and (3) "compensation" of the large diffusion current of the nobler metal by the application of a controllable counter e.m.f. to the recording galvanometer, so that the sensitivity may be increased to record the wave of the less minor metal (2, 5). All these methods have been used, although only the first two have real practical utility. The compensation method is attractive in principle, but has only limited practical application because large galvanometer oscillations persist when the nobler diffusion current is balanced out; such oscillations are only partly eliminated by "condenser damping", and it is not advisable to reduce their magnitude by simply increasing the drop time to a small value, because, as will be shown in a forthcoming paper, the diffusion current deviates significantly from the Ilkovič equation when the drop time is less than about one second.

Of the physical methods of separation, precipitation methods have been the most popular, but they suffer from two disadvantages which in certain cases are serious. In the first place, the separation of the minor constituents by coprecipitation is an ever-present hazard, and secondly the introduction of the precipitating reagents frequently complicates the analysis of the solution remaining.

The author found that electrodeposition at controlled cathode potential constitutes an excellent and generally applicable means of separating metals prior to polarographic analysis. The electrogravimetric separation and determination of metals by controlled potential have been developed to a high degree of practical utility, chiefly by the excellent investigations of Sand and his collaborators (6), but have not hitherto been applied in conjunction with polarographic analysis.

The separation of metals by electrodeposition is not without advantages. From a theoretical viewpoint the objection may readily be raised that an "absolutely complete" separation can never be attained, chiefly because of the exponential relation between the concentration of a metal ion and the potential of the cathode on which it is depositing, and the fact that the cathode potential must necessarily be limited to prevent the deposition of baser metals. However, this objection has only academic interest as far as the application of electroseparations to polarographic analysis is concerned, because complete separation is not required and it is only necessary to reduce the concentration of the interfering nobler metal to a value commensurate with that of the baser metal. Electroseparations also require special apparatus, which, however, is simple and easily available. These slight disadvantages are overshadowed by the fact that once the apparatus is assembled it can be applied to numerous separations, and by the further fact that separations are achieved rapidly with a minimum disturbance of the composition of the solution and without introducing extraneous reagents.

A mercury rather than a solid metal cathode has been used in the present investigation in order to capitalize on the fact that the current-voltage curves that are obtained polarographically with the dropping electrode provide all the information that is necessary about the potential to which the cathode must be

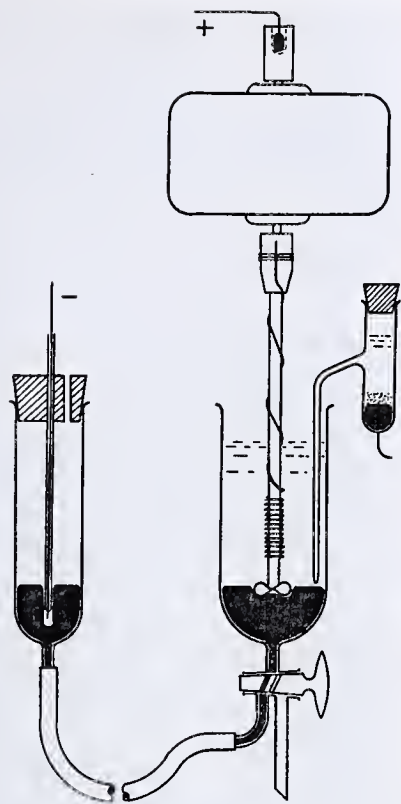


Figure 3. Cell for Electrolytic Separations at Controlled Potential with a Mercury Cathode

controlled to affect electroseparations, and thus a laborious, empirical establishment of optimum conditions is avoided. Although mercury cathodes have long been used in electrogravimetric analysis, apparently they have not heretofore been employed in determinations or separations at a controlled cathode potential, since no reference to such usage is to be found in the comprehensive bibliography (up to 1936) published by Böttger (1), nor in Sand's monograph (6) published in 1940.

The cell used is shown in Figure 3. It has a capacity of about 75 cc., and is provided with a two-way stopcock in the bottom for the introduction and withdrawal of the cathode mercury from the reservoir, and for withdrawing the solution after electrolysis. The mercury pool cathode has an area of about 10 sq. cm. and the mercury-solution interface (not merely the solution) is kept in rapid motion by the propeller-type glass stirrer.

The necessity of providing efficient stirring cannot be over-emphasized, because it is the factor which is chiefly responsible for the rate of deposition (current density) that can be obtained at a controlled potential. The stirring must be effective enough practically to eliminate concentration polarization at the mercury cathode, as otherwise the current density is more or less controlled by diffusion and is too small to permit separations in a reasonable time. It is not sufficient merely to stir the solution, however vigorously. The stirrer should be placed so that its blades are partly immersed in the mercury as shown in Figure 3, and the rate of stirring adjusted so that the interface is set in rapid circular motion without being so violent that drops of mercury are thrown about. The stirrer blades should be propeller-shaped, and so inclined that they impel downward rather than upward. Under these conditions of stirring the surface of the mercury undergoes vigorous but smooth churning and the entire pool revolves.

The cathode mercury or amalgam can be used repeatedly, provided that the metals previously deposited in it are more noble than the metal being separated—for example, mercury which contains copper can be used subsequently for the separation of bismuth, or if it contains copper and bismuth it may be used later in the separation of lead. Conversely, the mercury cannot be re-used when it contains a metal that is less noble than the metal being separated. For instance, mercury which has previously been used to separate bismuth or lead cannot later be used to separate copper, because at the relatively positive potential at which copper is deposited ( $-0.15$  to  $-0.17$  volt) the bismuth or lead undergoes anodic dissolution and contaminates the solution (see Figures 1 and 2). To avoid the use of excessive quantities of mercury and its frequent purification, it is convenient to keep the used mercury separated in three lots: one containing only dissolved copper and used only for copper separations, the second containing only copper and bismuth for copper and bismuth separations, and the third containing copper, bismuth, and lead. By keeping these three portions of mercury in contact with solutions of mercurous nitrate in dilute nitric acid the concentration of dissolved metals is kept small, and the dilute amalgams can be re-used indefinitely.

The cell was provided at first with a platinum anode, but this proved unsatisfactory for separations of copper from bismuth in an acidic tartrate medium because the bismuth was partially oxidized at the anode (probably to  $\text{Bi}_2\text{O}_3$ ) and precipitated. Attempts were made to prevent the oxidation of bismuth by adding small concentrations (0.05 *M*) of hydrazine sulfate or hydroxylamine hydrochloride to the solutions as anodic depolarizers, but, although oxidation of bismuth was thus eliminated, polarograms of the solutions after electrolysis showed spurious waves that interfered with the waves of the metals. A platinum anode is also undesirable because it introduces hydrogen ion into the solution and decreases its pH. These difficulties were finally circumvented by the use of a silver anode. This consists of No. 18 silver wire wrapped as a tight cylinder (area ca. 10 sq. cm.) around the stirrer shaft and spiraled up the shaft to the motor chuck, where it is held in place by a wrapping of copper wire. Electrical connection is completed by dipping the copper leading-in wire into a mercury pool in the top of the motor shaft. The bottom of the anode should be about 2 cm. above the stirrer blades to prevent it from short-circuiting the mercury cathode when the latter is stirred.

The solution to be electrolyzed is provided with a moderate concentration of chloride ion (0.1 to 0.2 *M*), so that the anode functions as a silver-silver chloride electrode, whose working potential is about 1 volt less positive than that of a platinum (oxygen) anode. Oxidation of bismuth and alteration of the pH of the solution are thus avoided, and the only effect of the anode reaction is the removal of chloride ion from the solution in amount equivalent to that of the metal deposited in the cathode.



Silver chloride is insoluble in an acidic tartrate medium and it deposits on the anode as an adherent coat. This coating reduces the effective area of the anode and increases the cell resistance (indicated by the necessity of increasing the total applied e.m.f. to maintain a constant cathode potential as electrolysis proceeds), and hence it is advisable to remove it after each experiment by electrolytic reduction from a dilute sulfuric acid solution in conjunction with a platinum anode.

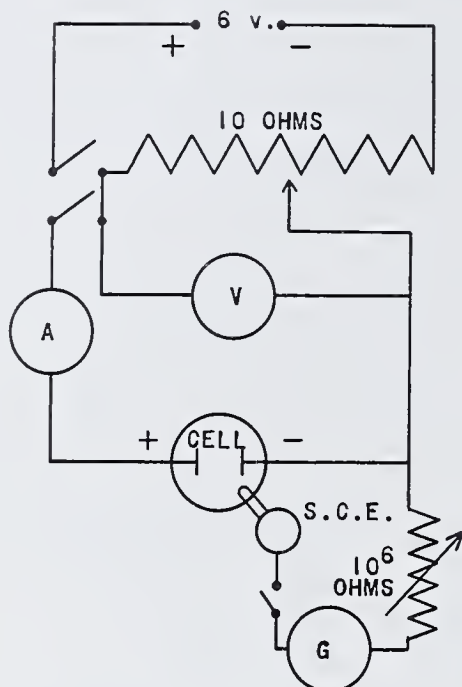


Figure 4. Electrical Circuit for Electrolytic Separations at Controlled Cathode Potential

A, ammeter (0 to 0.5 ampere). V, voltmeter  
G, galvanometer (0.01 microampere per mm.)  
S.C.E., saturated calomel reference electrode

The potential of the mercury cathode is measured continuously against a saturated calomel reference electrode during electrolysis, and controlled by changing the total e.m.f. applied to the cell. The salt bridge side arm from the calomel electrode is filled with a 3% agar gel saturated with potassium chloride, and its tip is constricted slightly to hold the gel in place. The tip of the bridge must be placed within about 1 mm. from the mercury cathode when the mercury is at rest (see Figure 3), so that the amount of ohmic potential drop that is necessarily included in the observed cathode potential will be negligible. The most satisfactory results are obtained when the tip is so adjusted that it actually becomes immersed slightly and trails in the mercury-solution interface when the stirrer is in motion, but it must not dip so deeply into the mercury that electrolytic contact with the solution is lost. Proper location of the tip can be tested by placing a dilute copper amalgam in the cell in contact with a copper solution, and, with the polarizing e.m.f. disconnected, comparing the observed cathode potential with and without stirring; the two readings should agree to 0.01 volt.

The electrical circuit is diagrammed in Figure 4. The e.m.f. applied to the cell is regulated by a single 10-ohm radio-type potentiometer-rheostat (General Radio Company, Type 333-A) and it is read on the voltmeter (0 to 6 volts). The voltmeter is a convenience rather than a necessity. A 0 to 0.5-ampere ammeter, graduated to 0.01 ampere, is used to measure the current.

The potential of the cathode *vs.* the saturated calomel reference electrode may be measured with a potentiometer, but it is much more convenient and amply accurate to measure it with a high-resistance voltmeter as recommended by Lassieur (3) and Sand (6). The author employed an improvised high-resistance voltmeter assembled from a critically damped Leeds & Northrup box-type galvanometer in series with a 0 to 5-megohm adjustable resistance. The galvanometer had a sensitivity of 0.01 microampere per mm., and thus the series resistance was about 1 megohm when it was adjusted to produce a full-scale (100-mm.) deflection with an impressed e.m.f. of 1 volt. The resistance of the instrument is so much larger than that through the salt bridge (ca. 700 ohms) that the error due to  $iR$  drop through the latter is only about 0.07% and hence negligible. Since the resistance of this voltmeter is so large it can be cali-

brated directly against a Weston standard cell (through tapping key) without damaging the latter. The instrument was calibrated to read from 0 to 1 volt, and the readings were precise and accurate to  $\pm 0.01$  volt.

Incidentally, the Sargent-Heyrovský polarograph can be used as a high-resistance voltmeter and thus made to serve a dual purpose. The instrument is set at maximum galvanometer sensitivity (ca. 0.003 microampere per mm.) and provided with an external resistance of about 3 megohms which is adjusted so that 100 mm. on the scale corresponds to 1 volt.

Gelatin must not be present during an electrolytic separation with the mercury cathode, because, as a result of its adsorption on the mercury surface, it causes the stirred mercury to disperse into droplets and erratic current readings result. The current density at a given value of the cathode potential was decreased considerably by even 0.01% of gelatin.

Care must be observed in discontinuing the electrolysis to prevent resolution of the deposited metal from the cathode. With the circuit still closed the mercury reservoir is lowered to drain the mercury from the cell, while the cathode potential is maintained constant at the value used during the deposition. When the mercury recedes to a point just above the stopcock the latter is turned through  $180^\circ$  C. to drain the last bit of mercury from the cell quickly. The loss of some solution during this last operation is of no consequence, because the subsequent polarographic analysis determines concentrations rather than absolute amounts of the metal ions remaining; the absolute amounts are computed from the concentrations and the volumetric stoichiometry involved in preparing the solutions for electrolysis.

Not the least of the advantages of electrolysis at a controlled potential is the fact that the current reading is a reliable criterion of the progress of the separation. In all the cases investigated the current finally dropped to less than 10 milliamperes from initial values ranging from 100 to 500 milliamperes, depending on the original concentration of the metal ion being deposited (compare Figure 6). Little is gained by continuing the electrolysis longer than 5 or 10 minutes after the current decreases to 10 milliamperes or less.

In the separations and polarographic determinations described below, the supporting electrolyte consisted of 0.4 *M* sodium tartrate plus 0.1 *M* sodium hydrogen tartrate plus 0.1 *M* sodium chloride (occasionally also 0.1 *M* sodium nitrate), and it is referred to simply as the "standard acidic tartrate supporting electrolyte." Electrolytic separations were performed at ambient room temperature in the presence of air, but the polarographic analyses were carried out at  $25^\circ$  C. after air was removed from the solution with nitrogen.

**SEPARATION OF COPPER.** Maximum rapidity of electrolytic separation of two metal ions requires that the potential of the

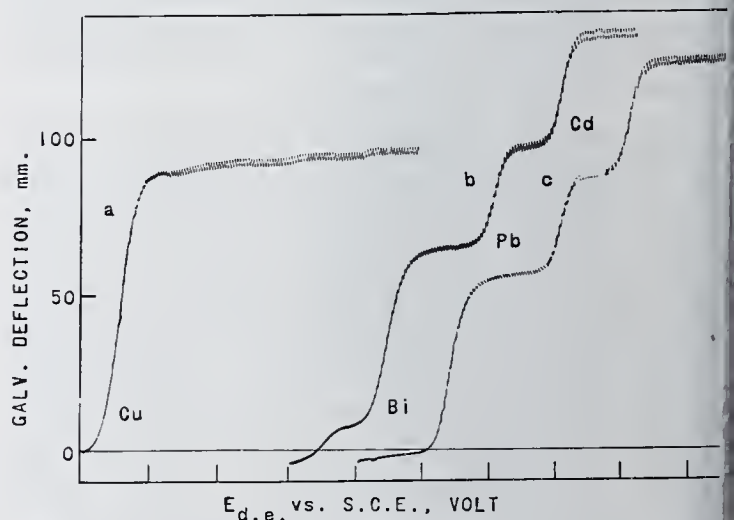


Figure 5. Analysis of Copper Group with Copper Predominant

Original solution contained, respectively, 100, 2.52, 2.00, and 2.32 millimoles copper, bismuth, lead, and cadmium, and polarograms were obtained at  $25^\circ$  C. with a standard acidic tartrate supporting electrolyte of pH 4.5 containing 0.005% gelatin. (a) 1 to 20 dilution of original solution, with galvanometer sensitivity 0.340 microampere per mm. (b) Residual solution from electrolysis of a 1 to 5 dilution of original solution with potential of mercury cathode between  $-0.13$  and  $-0.15$  volt *vs.* S.C.E. Galvanometer sensitivity 0.0680 microampere per mm. (c) Comparison solution containing same concentrations of bismuth, lead, and cadmium were present before electrolysis in solution b. Galvanometer sensitivity 0.01 microampere per mm. Each curve starts at 0 volt, and each voltage mark corresponds to an increment of 0.15 volt.



mercury cathode be maintained at a value sufficiently negative to yield the diffusion current of the first, but, to avoid codeposition, the potential must be more positive than that at which reduction of the second metal ion begins. Polarograms of solutions of copper and bismuth in the standard acidic tartrate supporting electrolyte indicate that the optimum potential range for the separation of copper from bismuth should be from  $-0.12$  to  $-0.16$  volt *vs.* the saturated calomel electrode, and this has been confirmed experimentally.

A typical experiment in which a large amount of copper was separated from small amounts of bismuth, lead, and cadmium is demonstrated by the polarograms in Figure 5. A stock solution, simulating the sort of solution that would result from the solution of the copper group sulfides in nitric acid, was prepared containing, respectively, 100, 2.52, 2.00, and 2.32 millimolar copper, bismuth, lead, and cadmium, as the nitrates in  $0.5 M$  nitric acid. Curve *a* in Figure 5 is a polarogram of a 20 dilution of the stock solution in the standard acidic tartrate supporting electrolyte. The waves of bismuth, lead, and cadmium are scarcely detectable in the presence of the 40-fold excess of copper.

Fifty cubic centimeters of a  $1/5$  dilution of the stock solution—i.e.,  $0.02 M$  in respect to copper—in the standard acidic tartrate solution were subjected to electrolytic separation as described above with the potential of the mercury cathode maintained between  $-0.13$  and  $-0.16$  volt. The current-time curve for this separation is shown in Figure 6, and is typical of all those obtained in other cases. Following an initial rapid decrease during the first 3 minutes, during which a steady state electrolysis was established, the current remained constant to about 10 minutes, and then decreased rapidly and finally gradually approached zero. The solution became colorless after 10 minutes and the electrolysis was stopped after 40 minutes—i.e., 10 minutes after the current had fallen below 10 milliamperes. A 20-cc. sample of the residual solution was treated with 0.5 cc. of a 0.2% gelatin solution, and its polarogram was recorded as curve *b* in Figure 5. The galvanometer sensitivity for curve *b* is approximately five times greater than that for curve *a*, and the original concentrations of the metal ions were four times as great.

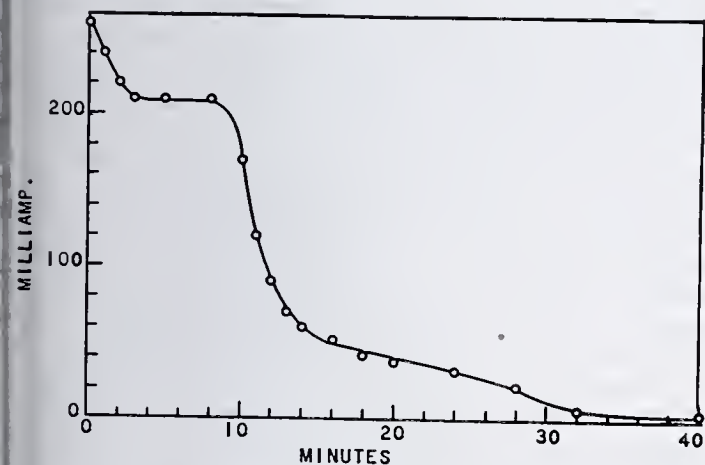


Figure 6. Typical Current-Time Curve

Obtained during electrolysis of an acidic tartrate solution of pH 4.5 containing 20 millimolar copper, 0.50 millimolar bismuth, 0.40 millimolar lead, and 0.465 millimolar cadmium. Potential of mercury cathode between  $-0.13$  and  $-0.16$  volt *vs.* S.C.E.

In addition to the well-defined waves of bismuth, lead, and cadmium, a small wave due to residual copper is present in the polarogram of the solution after electrolysis (curve *b* in Figure 5). This small wave corresponds to only 0.09 millimolar copper compared to 20 millimolar before electrolysis. In other words, electrolytic separation was 99.5% effective, which is satisfactory, considering the fact that the half-wave potentials of copper and bismuth differ by only 0.20 volt. Obviously the small amount of residual copper does not interfere with the waves of the other metals.

To establish the fact that the other metals, particularly bismuth, were not codeposited with the copper during the electrolytic separation, a comparison solution was prepared in the standard acidic tartrate medium containing the same concentrations of bismuth, lead, and cadmium—i.e., 0.504, 0.400, and 0.464 millimolar—as were present originally in the solution before electrolysis. The polarogram of a 20-cc. sample of this com-

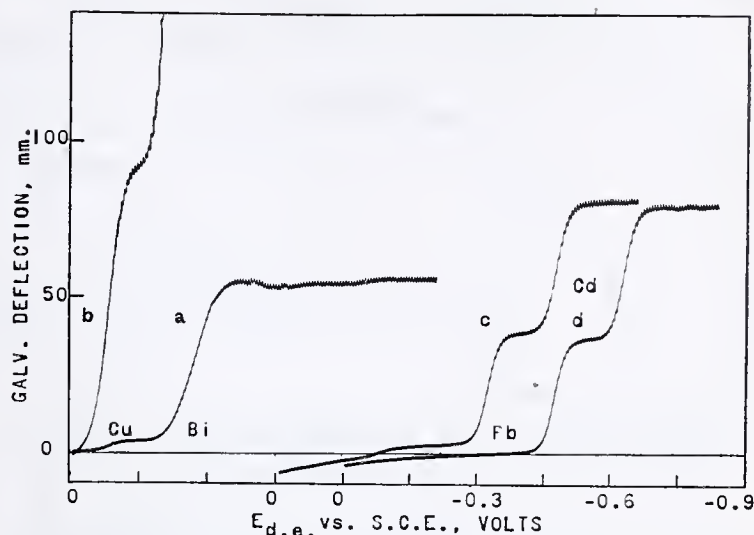


Figure 7. Analysis of Copper Group with Bismuth Predominating

(a) Original solution containing 1.04 millimolar copper, 10.07 millimolar bismuth, 0.400 millimolar lead, and 0.465 millimolar cadmium, in acidic tartrate supporting electrolyte of pH 4.5 containing 0.005% gelatin. Galvanometer sensitivity 1.70 microamperes per mm. (b) Polarogram of solution *a* repeated with galvanometer sensitivity 0.0680 microampere per mm. (c) Solution remaining after electrolysis of solution *a* with potential of mercury cathode between  $-0.35$  and  $-0.40$  volt *vs.* S.C.E. Galvanometer sensitivity 0.0680 microampere per mm. (d) Comparison solution containing same concentrations of lead and cadmium as in solution *a*. Galvanometer sensitivity 0.0680 microampere per mm.

parison solution plus 0.5 cc. of 0.2% gelatin is shown as curve *c* in Figure 5. A comparison of the waves of bismuth, lead, and cadmium, in curves *b* and *c* shows that no appreciable amounts of these metals were codeposited with the copper. It is evident that successful results could be obtained with a larger proportion of copper to bismuth, lead, and cadmium than in this experiment, and it should be possible to determine considerably less than 1 mole % of any of the three other metals in copper and its compounds.

When, in the separation of copper from bismuth, the cathode potential is allowed to exceed  $-0.16$  volt, bismuth is codeposited to an extent dependent on how greatly this limiting potential is exceeded. For example, in another experiment identical with the above in all respects except that the cathode potential was maintained at  $-0.18$  volt, 14% of the bismuth was lost.

In the separation of copper from lead the cathode potential may be as large as  $-0.40$  volt, which permits a larger current and correspondingly shorter time of electrolysis (for a given amount of copper) than in the separation from bismuth. Conditions are even more favorable in the separation of copper from cadmium.

At a given value of the cathode potential the initial current is approximately proportional to the concentration of the metal ion being deposited, and consequently the time required for a complete electrolysis is roughly the same regardless of the concentration. For example, the separation of copper at a cathode potential of  $-0.16$  volt required 30 to 40 minutes when the concentration of copper ranged from a few millimolar up to  $0.05 M$ .

**SEPARATION OF BISMUTH.** The analysis of the copper group when bismuth predominates is exemplified by the polarograms in Figure 7.

A stock solution was prepared containing 1.04 millimolar copper, 10.07 millimolar bismuth, 0.400 millimolar lead, and 0.465 millimolar cadmium, in the standard acidic tartrate supporting electrolyte. Curve *a* in Figure 7 is a polarogram of a 20-cc. sample of this solution to which 0.5 cc. of 0.2% gelatin was added. The copper wave is clearly discernible before the large bismuth wave, but the lead and cadmium waves are barely detectable. The wave of this relatively very large concentration of bismuth shows a small rounded maximum, and hence it would have been better practice to dilute the solution five- or tenfold before recording its polarogram. In order to magnify the copper wave for accurate measurement, the galvanometer sensitivity was increased by a factor of 25 and curve *b* was recorded.



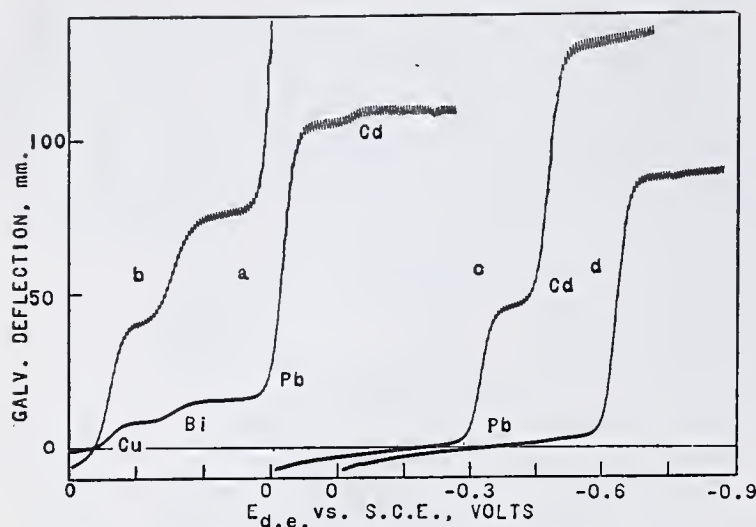


Figure 8. Analysis of Copper Group with Lead Predominating

Original solution contained 5.05 millimolar copper, 2.52 millimolar bismuth, 50.3 millimolar lead, and 2.32 millimolar cadmium. Polarograms obtained at 25° C. from acidic tartrate supporting electrolyte of pH 4.5 containing 0.005% gelatin (a) 1 to 20 dilution of original solution, with galvanometer sensitivity 0.170 microampere per mm. (b) Polarogram of solution a repeated with galvanometer sensitivity increased to 0.0340 microampere per mm. (c) Solution remaining after electrolyzing a 1 to 5 dilution of the original solution, with potential of mercury cathode between -0.54 and -0.56 volt vs. S.C.E. Galvanometer sensitivity 0.0340 microampere per mm. (d) Comparison solution containing same concentration of cadmium as solution c before electrolysis. Galvanometer sensitivity 0.0340 microampere per mm.

A 50-cc. sample of the original solution was electrolyzed as described previously to remove the copper and bismuth. The electrolysis was started with the potential of the mercury cathode at -0.35 volt, and was gradually allowed to increase to -0.40 volt and then held constant at this value until the electrolysis was complete (compare polarograms of bismuth and lead). The electrolysis was continued for 40 minutes, during which time the current dropped from an initial value of 150 to 3 milliamperes. A 20-cc. sample of the residual solution was then treated with 0.5 cc. of 0.2% gelatin, and curve c in Figure 7 was recorded at a relatively great galvanometer sensitivity. Only a trace (0.02 millimolar) of bismuth remained in the solution, and the separation was 99.8% complete.

Curve d (Figure 7) is the polarogram of a comparison solution containing the same concentrations of lead and cadmium (0.400 and 0.465 millimolar) as were present in the original solution. By comparing curves c and d it is evident that no detectable amounts of lead or cadmium were lost during the deposition of the copper and bismuth.

The separation of bismuth from lead and cadmium is so complete that it should be possible to determine a few tenths of a per cent of the latter metals in bismuth and its compounds.

**SEPARATION OF LEAD.** A typical example of the analysis of the copper group with lead predominating is furnished by the polarograms of Figure 8.

A stock solution was prepared containing 5.05 millimolar copper, 2.52 millimolar bismuth, 50.3 millimolar lead, and 2.32 millimolar cadmium in 0.5 N nitric acid. A 1/20 dilution of this solution was prepared in the standard acidic tartrate medium, a 20-cc. sample was treated with 0.5 cc. of 0.2% gelatin, and polarograms a and b of Figure 8 were recorded. Curve a at the lower galvanometer sensitivity shows the waves of all four metals distinctly. Curve b was recorded at an increased galvanometer sensitivity to magnify the waves of copper and bismuth to measurable magnitudes.

A 50-cc. sample of a 1/5 dilution of the stock solution in the acidic tartrate supporting electrolyte was prepared and electrolyzed to remove copper, bismuth, and lead. When this solution was prepared a considerable proportion of the lead slowly precipitated as coarse crystals of lead hydrogen tartrate. The precipitate caused no trouble and gradually dissolved as electrolysis proceeded. The potential of the mercury cathode was maintained between -0.54 and -0.56 volt, the optimum values indicated by the polarograms of lead and cadmium. The initial current was 100 milliamperes, it decreased to 8 milliamperes after 28 minutes, and the electrolysis was discontinued after 35 minutes. A 20-cc. sample of the residual solution was treated with 0.5 cc. of 0.2% gelatin, and curve c of Figure 8 was recorded. This polarogram shows that the residual solution contained a small amount of lead (0.24 millimolar), but since its wave is smaller than that of the cad-

mium it does not interfere with the measurement of the latter. By continuing the electrolysis for a somewhat longer time a more complete separation could doubtless be obtained, but this will be necessary only when the concentration of cadmium is very small.

Curve d in Figure 8 is a comparison solution containing the same concentration of cadmium (0.465 millimolar) as in the original solution, and by comparing this curve with curve b it is evident that no cadmium was lost during the deposition of the lead.

#### CONCLUDING REMARKS

In the application of the foregoing methods it is most convenient if the size of sample taken for analysis corresponds to not less than about 5 mg. nor more than about 500 mg. of any member of the copper group in a final volume of 100 cc.

Standard procedures may be used to prepare a solution of the sample, to remove silver and mercurous ions as the chlorides to precipitate the hydrogen sulfide group, and to separate the copper and tin groups. The procedures described by Swift (8) are particularly suitable for these preliminary separations.

The washed copper group sulfides are dissolved in dilute nitric acid, and, after boiling to remove hydrogen sulfide and oxide of nitrogen, the solution is diluted to a known volume—e.g. 100 cc. An aliquot portion of this "copper group solution" is then made up to a known volume in the standard acidic tartrate medium with 0.005% gelatin (after either partial neutralization or addition of dilute nitric acid as required to provide a ratio of tartrate to hydrogen tartrate ion of 4 to 1). Polarograms of this solution are then recorded, and from those waves that are of measurable height the corresponding concentrations are computed with the aid of the known diffusion current constants already described (4).

Those electrolytic separations dictated by the first polarogram are then carried out and the residual solutions are analyzed as described above. It is most convenient to use separate aliquot portions of the original copper group solution in the acidic tartrate supporting electrolyte for these separations. It is also advisable to perform separations of the nobler constituents, and polarographically test for the baser metals in the residual solutions, even though waves of the latter were not observed in the original polarogram, because traces of a baser metal may be completely masked by large amounts of a nobler metal (see Figure 5). Furthermore, such separations should be performed one at a time. For example, an original polarogram might show large copper and lead waves but no detectable bismuth wave if only a very small amount of bismuth were present. In such a case the cathode potential during the separation should not be more negative than -0.16 volt, so that only copper will be removed and any bismuth can be detected and determined from the polarogram of the residual solution.

The combination of electrolytic separations with polarographic analysis is obviously capable of general application in a systematic scheme of metal analysis, and it should prove useful for various special alloy analyses. Further examples of the utility of the method will be described in forthcoming papers.

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# Determination of Sulfur Dioxide in Dehydrated Foods

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Details are presented of a rapid direct titration method for determining sulfur dioxide in dehydrated foods. The reliability of the method has been established by recovery of added sulfur dioxide and by comparison with distillation and polarographic methods.

AS A control measure in the application of sulfite solutions to vegetables intended for dehydration, a simple method for determining the sulfur dioxide content has become necessary. The method proposed here has been designed for inspection and field work with a minimum of equipment but is also well adapted to laboratory research. It has been compared with other chemical methods and also the polarographic method.

The methods of Monier-Williams (10), an official A.O.A.C. method, and of Nichols and Reed (11) involve distillation of the sulfur dioxide into neutral hydrogen peroxide or standard iodine solution. Simultaneously with the publication of the Monier-Williams report, there appeared a series of reports on the determination of sulfur dioxide by a British committee and other contributors (2). The committee method, which includes rapid distillation of the sulfur dioxide into an iodine solution, is of limited application. The Nichols and Reed method, employing distillation, has been widely used in California on dried fruits.

The distillation methods are time-consuming and not well adapted to field work. In addition, with cabbage, onions, and other vegetables having a high volatile-sulfur content, abnormally high values are obtained on the unsulfited controls when the distillate is absorbed in iodine.

To eliminate many of the objectionable features of the distillation methods, direct titration of an aqueous extract either with or without preliminary clarification has been proposed. Liberation of bound sulfur dioxide by alkaline treatment forms the basis of the method, first described by Ripper (12). One may use the double titration technique in which one sample is treated for total reducing substances and the other is treated to remove sulfur dioxide from the reaction. It can be oxidized to sulfate, or removed by formation of an addition complex. The latter method is preferable, in the authors' opinion, because there is less possibility of drastic change in the other constituents of the food, and the two titrations differ only with respect to one component—sulfur dioxide.

Two recent papers, one by Iokhel'son and Nevstrueva (3) and another by Bennett and Donovan (1), describe direct titration methods. In the former the dehydrated food material is treated with alkali solution to liberate the "bound" sulfur dioxide; the extract is clarified and then titrated with iodine to yield the total reducing substances. A similar sample is treated with formaldehyde to react with the sulfur dioxide present. Liberation of this sample yields reducing substances other than sulfur dioxide, which is then calculated from the difference between the two titrations. This method, involving clarification, is time-consuming. The Bennett and Donovan procedure was developed for citrus juices which are used directly without extraction or clarification, and acetone is used instead of formaldehyde. Unfortunately, data on proper conditions for wider applicability of the method are not included.

The method described here is an adaptation of these direct titration procedures. The specific optimum conditions have been determined for each step of the analysis. For convenience, the modified Bennett-Donovan procedure is referred to as the direct titration method.

## PREPARATION OF SAMPLE

Considerable care must be used in preparing the sample. A representative sample must be ground or subdivided into suffi-

ciently fine particles so that the solutions used will leach out all the sulfur dioxide. In the distillation methods the boiling process effectively disintegrates the tissue, even though relatively large pieces are placed in the still. The direct titration methods depend upon leaching action without heating and require the sample to be finely subdivided before analysis. Preferably a mill such as the Wiley mill with a 2-mm. screen should be used, in which the sample is ground until practically all has passed through the 2-mm. screen. A food grinder designed for preparing vegetable purees can be used, provided certain conditions as to load and time of grinding are adhered to, so that practically all the material passes a 10-mesh screen and at least 60% passes a 20-mesh screen. For the samples reported in this paper adequate subdivision was obtained by 1.5 minutes of grinding at high speed with a 30-gram charge of dehydrated shredded cabbage or a 100-gram charge of dehydrated diced carrots or potatoes. If large pieces of material are left unground, low results are obtained in the assay.

Dried fruits have physical characteristics different from those of dried vegetables and must be handled differently. By passage through a kitchen food chopper, followed by soaking and grinding in a food blender, it is possible to prepare uniform, finely divided suspensions of dried fruits for analysis. The presence of any lumpy material not finely divided will generally yield low analytical results.

## ALKALINE LIBERATION OF BOUND SULFUR DIOXIDE

For each assay an 8-gram sample of the ground dehydrated material was suspended in 400 ml. of water, 5 ml. of 5 *N* sodium hydroxide were added, and the mixture was allowed to stand for 20 minutes. The amount of alkali needed is not critical in itself, but must be accurately measured. The quantity indicated gives a mixture sufficiently alkaline to release the bound sulfur dioxide, while four times as much yields the same result. The length of time necessary to liberate the sulfur dioxide was determined by comparing results over periods up to 60 minutes (Table I). No significant variation in sulfur dioxide liberation was found after 10 minutes up to 60 minutes. Twenty minutes was chosen as a convenient time.

Table I. Effect of Time of Alkali Treatment on Liberation of Sulfur Dioxide

Length of Alkali Treatment Min.	Sulfur Dioxide		
	Cabbage <i>P.p.m.</i>	Carrots <i>P.p.m.</i>	Potatoes <i>P.p.m.</i>
0 (no alkali treatment)	2020	1880	1410
1	2520	2060	
5	2860	2260	1960
10	3020	2380	2100
20	3060	2400	2040
30	3060		
40	3060	2400	2020
50	3040		
60	3120	2400	2040

## FORMATION OF ACETONE-SULFUR DIOXIDE COMPLEX

The effects of pH, acetone concentration, and reaction time on the formation of the complex were determined.

To study the effect of pH on the formation and stability of the complex, 8-gram samples in 400 ml. of water were treated with alkali for 20 minutes, acidified to different pH levels, treated with 40 ml. of acetone, allowed to stand 10 minutes, and titrated with 0.05 *N* iodine solution. Minimum iodine titers represent reducing substances other than sulfur dioxide and indicate maximum binding of the sulfur dioxide. Table II shows that maximum binding occurs in cabbage and carrots between pH



Table II. Effect of pH on Stability of Acetone Complex

pH	0.05 N Iodine Required		
	Dehydrated cabbage ml.	Dehydrated carrots ml.	Dehydrated potatoes ml.
1.0	5.20	1.50	0.60
1.5	4.40	...	...
2.0	4.00	0.90	0.40
2.6	4.20	0.90	...
3.0	4.20	0.90	0.40
3.5	4.45	1.00	...
4.0	4.50	1.00	0.40
5.0	5.20	3.10	1.60
6.0	8.40	4.00	7.00

2 and 3, whereas in potatoes the range is extended to pH 4. For safe general applicability, the range of pH 2 to 3 was chosen.

Kolthoff and Furman (4, 7) observe that the addition compound is unstable in strongly acid solution, and state that the optimal pH for addition is about that of a bisulfite solution—that is, 4.0. Their discussion is concerned with the determination of aldehyde or ketone in the presence of excess sulfite, whereas the reverse is the present objective. Experimentally, with the foods tested, a pH range from 2 to 3 was found most suitable. This range offers the additional advantage of a good end point; the starch-iodine end point is less satisfactory with an excess of acetone at pH 4.

The same pH range was found to be optimum for analysis of dried fruits. It is necessary, therefore, to maintain this pH during the acetone treatment and titration. A similar range is desirable for the formaldehyde complex.

To determine the amount of acid required to neutralize the alkaline digestion mixture and yield a pH in the proper range, buffer curves were drawn in which pH was plotted for the mixture to which successive increments of 5 N acid were added. Dehydrated cabbage, carrots, and potatoes all gave the same curve, as might be anticipated under these conditions, and required 7.5 ml. of 5 N hydrochloric acid. Dehydrated apples, apricots, and peaches gave a curve slightly different from that obtained with vegetables and required only 6 ml. of the 5 N acid. The amount of acid required to bring the alkaline digestion mixture to the pH range of 2 to 3 should be determined for each commodity. Because the optimum pH range is in a region where the pH is affected considerably by added acid, it is necessary to measure the alkali and acid accurately with pipets or dispensing burets rather than with graduated cylinders.

Concentrations of acetone up to 33% by volume have been recommended for binding the sulfur dioxide (1, 8). In high concentration, acetone interferes with the development of the color of the starch-iodine end point. By varying the amount of acetone used, it has been found that quantitative binding of the sulfur dioxide occurs under the conditions of the analysis at concentrations of acetone of 10% by volume or even slightly less. With 10% acetone present, satisfactory end points can be obtained, provided sufficient starch is added.

In the pH range indicated, the reaction between the acetone and the sulfur dioxide is complete in approximately 5 minutes. Standing for longer periods, up to 2 hours, was without further effect. For convenience of operation when several samples were being run, a 10-minute interval was adopted.

Many substances will react with sulfur dioxide to form stable complexes. Formaldehyde does not interfere with the color at the starch end point and yields satisfactory results with some commodities. In some cases the end point is easier to follow with formaldehyde than with acetone. However, formaldehyde consistently yields results 5 to 10% higher with cabbage than does acetone.

As noted by Kolthoff and Furman (4, 7) the analysis hinges upon two factors: the dissociation constants,  $K$ , of the salts of the bound sulfurous acid and the speed of establishment

of equilibrium. The  $K$  for the formaldehyde complex is theoretically preferable ( $1.2 \times 10^{-7}$  at 25° C., compared with  $4 \times 10^{-3}$  for acetone).

The working conditions are in the range  $10^{-3}$  M for bisulfite and  $M$  for acetone. It is thus possible to calculate that a titration error caused by incomplete binding is of the order of 0.4%. However, as the titration approaches completion, the equilibrium has been destroyed, and differences in the speed of the reverse reaction may be responsible for the divergences between acetone and formaldehyde. This would imply that the formaldehyde value would be more nearly correct, but this in turn would suggest an error in the Monier-Williams value. The reasoning is not entirely satisfactory because the divergence is not uniform for all commodities but is most pronounced with cabbage. Glyoxal, which is now commercially available in a 3 to 40% solution, may prove satisfactory, although the details have not been worked out.

Because of acetone interference at the starch end point, other indicators were tried. Methylene blue was found satisfactory but no better than the starch as finally used. By using a large amount of starch, 10 ml. of 1% solution per titration, and a minimum amount of acetone, about 10% by volume, it was possible to obtain satisfactory end points.

Table III. Replicate Analyses of Samples

Dehydrated Samples	P.p.m. SO <sub>2</sub>	Average
Cabbage A	413, 433, 352, 413, 372	397
Cabbage B	1180, 1180, 1260, 1260, 1260, 1240	1230
Cabbage C	1340, 1360, 1320, 1240, 1280	1308
Cabbage D	3060, 3060, 3060, 3040, 3120	3068
Cabbage E	3320, 3260, 3360, 3280, 3340	3312
Carrots A	2380, 2400, 2400, 2400, 2400	2396
Carrots B	2320, 2240, 2240, 2220, 2300	2264
Potatoes A	165, 155, 145, 155, 175	159
Potatoes B	2085, 2015, 2170, 2000, 2085	2071

#### ANALYTICAL PROCEDURE

The determination is best carried out in a 600-ml. tall-form beaker with the aid of a slow-speed stirrer carrying a propeller with broad blades having a steep pitch. With this type of stirrer, thorough mixing is obtained without beating air into the mixture. If a stirrer is not available the determination can be carried out in a 1-liter Erlenmeyer flask or even a quart fruit jar, which is shaken during the analysis.

Two 8-gram samples ( $\pm 10$  mg.) of the dehydrated product are transferred to the titration vessels; 400 ml. of water and 5 ml. of 5 N sodium hydroxide are added to each and the mixtures are stirred. After 20 minutes the mixtures are acidified with 5 N hydrochloric acid—7.5 ml. for cabbage, carrots, potatoes, or 6 ml. for dried apricots, apples, or peaches. To one of the samples 40 ml. of acetone are added, while the other sample is titrated at once with 0.05 N iodine solution using 1 ml. of 1% soluble-starch solution. It is important that the acidified sample be titrated at once before recombination occurs. The end point is reached when, with vigorous stirring or shaking, a blue color flashes throughout the entire mixture and persists for a few seconds. The acetone-treated mixture is titrated similarly after standing for 10 minutes.

The end points in the titrations are fleeting. In the samples containing acetone this is particularly true because the compound continuously yields a small amount of sulfur dioxide by decomposition. When no acetone is present, the end point is more persistent but even then it is not permanent. With these so-called flash end points, quantitative results can be obtained. However, in cases where the available equipment will not grind the sample fine enough, it is possible arbitrarily to standardize the method against the Monier-Williams method by choosing a more permanent end point for the titration carried out in the absence of acetone.

Reagent blanks must be determined by a similar set of titrations.



Table IV. Recovery of Sulfur Dioxide Added to Dehydrated Sulfited Cabbage and Carrot Suspension				
SO <sub>2</sub> Originally Present, A Mg.	SO <sub>2</sub> Added Mg.	SO <sub>2</sub> Found (Total), B Mg.	SO <sub>2</sub> Recovered, B - A Mg.	Recovery %
Cabbage				
10.33	19.21	26.82	16.49	85.8
10.33	19.21	27.04	16.71	87.0
10.33	19.21	27.26	16.93	88.1
				Av. 87.0
10.33	37.80	45.10	34.77	92.0
10.33	37.80	45.30	34.97	92.5
				Av. 92.2
10.33	75.15	80.05	69.72	92.8
10.33	75.15	80.40	70.07	93.3
				Av. 93.0
Carrots				
18.02	19.91	37.30	19.28	96.8
18.14	19.93	37.46	19.32	96.8
18.14	19.93	37.15	19.01	95.4
				Av. 96.3
18.02	39.95	56.50	38.48	96.3
18.02	39.95	56.40	38.38	96.0
18.14	38.75	56.05	37.91	97.8
				Av. 96.7
18.02	78.30	95.28	77.26	98.7
18.02	78.30	94.90	76.88	98.2
18.14	77.15	94.00	75.86	98.4
				Av. 98.4

ons without food material and subtracted from the corresponding titration values. The difference between the two corrected titers multiplied by 200 yields sulfur dioxide in parts per million. For material with a high starch content, such as potatoes, the titration is continued until the mixture has developed a deep blue color. During the titration some iodine is adsorbed on the small solid potato particles, causing them to be blue-black. This is removed slowly and represents only a very small amount of the total iodine used. It does, however, impart a gray cast to the mixture which is not to be mistaken for the true end point. By addition of iodine until a deep blue color is reached, correct and consistent values are obtained.

ANALYTICAL RESULTS

The reproducibility of results with the proposed method was checked by repeated assays of sulfited dehydrated cabbage, carrot, and potato samples (Table III). Accuracy was checked both by recovery of added sulfur dioxide and by comparison with other methods. In addition to the methods of Monier-Williams and Nichols and Reed, which were used for comparison, check determinations were carried out with the aid of the dropping mercury electrode (5, 6). To determine the recovery of added sulfur dioxide, standard amounts of sodium metabisulfite solution were added to suspensions of dehydrated sulfited cabbage and carrots. These samples were analyzed before and after the addition of the sulfite solution. Very erratic results were obtained under these conditions, the deviations being considerably greater than those encountered in actual analytical practice. Plant material contains compounds that exert a protective action and inhibit the destruction of sulfur dioxide by oxidation or other reactions. Samples of sulfured commodities ready for analysis contain the sulfur dioxide in a form which is protected and not readily susceptible to loss in handling; in samples to which sulfite solutions have not been added the sulfur dioxide is stabilized only after an appreciable lapse of time. It was found that the addition of 0.1% of sodium pyrophosphate decahydrate to the sodium metabisulfite solution led to consistent recovery data. The literature on the inhibitors of sulfur dioxide destruction, other than sodium pyrophosphate, has been reviewed by Monier-Williams (10) and Mitchell, Pitman, and Nichols (9).

Table IV lists the data obtained on the recovery of sodium metabisulfite solution stabilized with sodium pyrophosphate when added to dehydrated cabbage and carrots. In each case, an 8-gram sample was taken and the regular analysis performed except that the indicated amounts of metabisulfite solution were added to 400 ml. of suspension before addition of alkali. The data in Table IV show fair recovery in cabbage and good recovery in carrots. Even though these commodities were both sulfured before dehydration and contained appreciable sulfur dioxide, in the case of cabbage there was a consistent loss of added sulfur dioxide. This loss probably would not appear during routine analysis of samples which have been dehydrated after sulfuring, because the loss would have occurred during the processing. Representative data comparing the direct titration method with the distillation methods for several commodities are given in Table V.

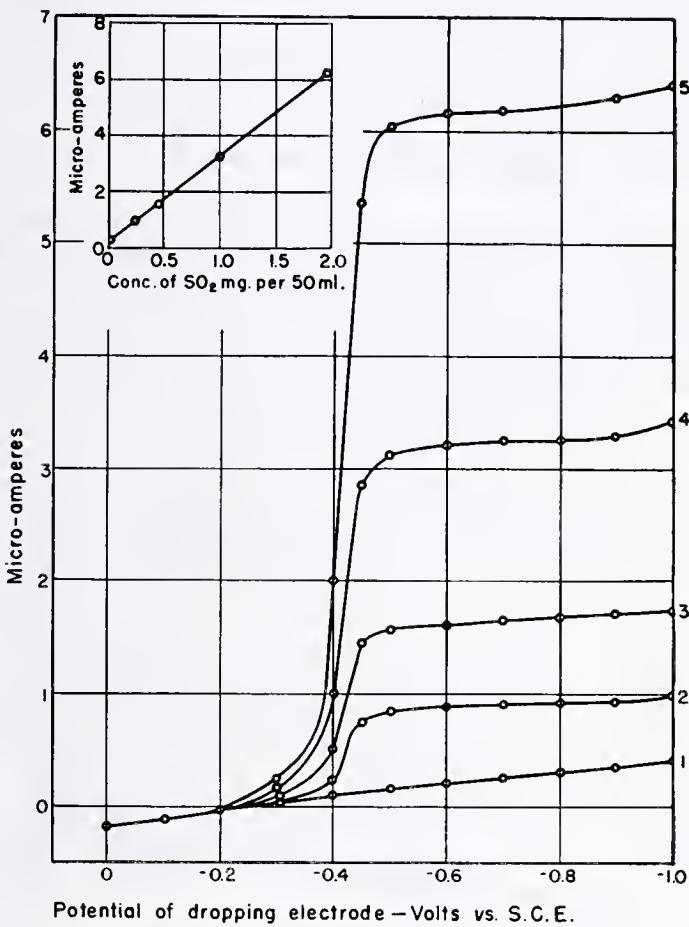


Figure 1. Measurements with Dropping Mercury Electrode  
Supporting electrolyte: (1) 0.1 N hydrochloric acid; (2) plus 0.243 mg. of SO<sub>2</sub> per 50 ml.; (3) plus 0.486 mg. of SO<sub>2</sub> per 50 ml.; (4) plus 0.972 mg. of SO<sub>2</sub> per 50 ml.; (5) plus 1.944 mg. of SO<sub>2</sub> per 50 ml.

Table V. Comparison of Methods of Sulfur Dioxide Analysis						
Commodity	Direct Titration	Monier-Williams		Nichols-Reed		
		P.p.m. SO <sub>2</sub>				
Dehydrated cabbage A, not sulfited	100	...	60	68	320	520
Dehydrated cabbage B, sulfited	372	413	464	468	640	840
Dehydrated cabbage C, sulfited	2310	2450	2560	2480	2632	2570
Dehydrated cabbage D, sulfited	4490	...	4580	4570	4500	4340
Dehydrated carrots A, not sulfited	0	0	20	20	0	0
Dehydrated carrots B, sulfited	2520	2620	2610	2690	2380	2420
Dehydrated carrots C, sulfited	6260	6220	6390	6460	6300	6320
Dehydrated onions A, not sulfited	86	86	153	121	850	840
Dehydrated onions B, sulfited	445	414	759	644	1490	1550
Dehydrated potatoes A, not sulfited	20	20	40	40	0	0
Dehydrated potatoes B, sulfited	42	83	142	146	0	60
Dehydrated potatoes C, sulfited	249	314	300	322	180	220
Dehydrated potatoes D, sulfited	853	687	752	732	660	660
Dehydrated potatoes E, sulfited	2160	2225	2295	2305	2090	2100
Dried apples sulfited	3055	...	2960	2930	2615	2555
Dried apricots A, sulfited	1605	...	1663	1700	1520	1500
Dried apricots B, sulfited	884	884	914	909	680	886
Dried peaches sulfited	1685	...	1705	1735	1600	1555



## MEASUREMENTS WITH DROPPING MERCURY ELECTRODE

Most of the methods of determining sulfur dioxide content depend upon general oxidation or neutralization reactions and are not specific. To obtain data specific for the molecular species involved, a polarographic method employing the dropping mercury electrode was used. Kolthoff and Miller (6) have reported results on pure aqueous solutions of sulfurous acid.

A simple manual dropping mercury electrode was used (5). A number of capillaries were prepared by drawing out capillary tubing with a 0.25-mm. bore. One having a drop time of about 2 seconds was finally chosen and used in all these studies, since it gave the maximum values of diffusion current that still maintained approximate linearity with concentration. Measured at  $-0.6$  volt in  $0.1 N$  hydrochloric acid, the values for use in the Ilković equation (5) were as follows:

$$t = 2.33 \text{ sec.}$$

$$m = 1.83 \text{ mg. sec.}^{-1}$$

$$m^{2/3}t^{1/6} = 1.72 \text{ mg.}^{2/3} \text{ sec.}^{-1/2}$$

Preliminary experiments led to the choice of  $0.1 N$  hydrochloric acid as the supporting electrolyte. To calibrate the apparatus,  $0.1 N$  hydrochloric acid was introduced into the electrolysis cell and swept free of dissolved oxygen with a stream of nitrogen. The gas flow was then diverted to flow over the surface of the solution, aliquots of dilute sodium bisulfite solution were introduced to make a final volume of 50 ml., the mixture was stirred carefully to avoid incorporating oxygen, and the current-voltage data were recorded.

Representative data are plotted in Figure 1, which includes the current-voltage curves for pure aqueous solutions of sodium bisulfite in  $0.1 N$  hydrochloric acid and, in the upper left corner, a graph showing the relation between the current (sum of diffusion and residual currents) at  $-0.6$  volt and the concentration of sulfur dioxide.

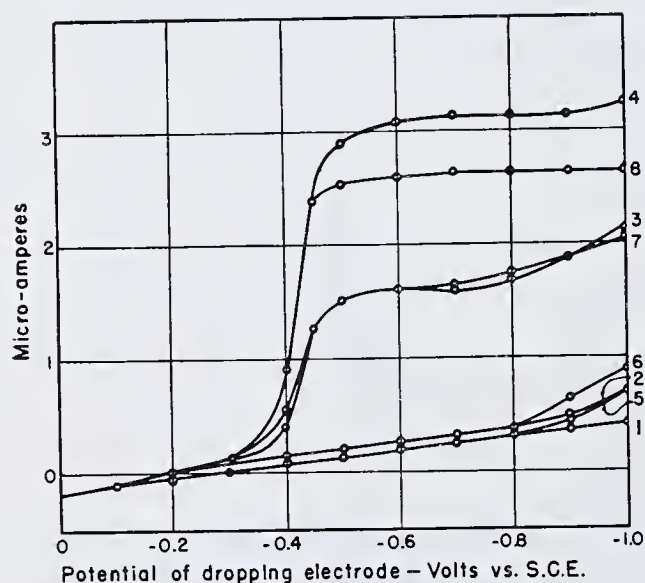


Figure 2. Measurements with Dropping Mercury Electrode

1. Supporting electrolyte,  $0.1 N$  hydrochloric acid
2. Unsulfited cabbage
3. Unsulfited cabbage plus  $0.486$  mg. of  $\text{SO}_2$  per 50 ml.
4. Sulfited cabbage
5. Sulfited cabbage plus acetone
6. Unsulfited carrots
7. Unsulfited carrots plus  $0.486$  mg. of  $\text{SO}_2$  per 50 ml.
8. Sulfited carrots

Three drops of 1% gelatin solution were added to suppress maxima in pure solutions. Maximum suppressors were not necessary in suspensions of vegetable material. All voltages were measured with a saturated calomel half-cell as the reference electrode. Typical curves obtained with cabbage and carrot suspensions are given in Figure 2.

As shown in the figures, the half-wave potential for sulfur dioxide is the same in pure aqueous solutions as in cabbage or carrot suspensions. Under the conditions used there were no interfering waves in the dehydrated vegetable suspensions. Good

Table VI. Sulfur Dioxide Found by Dropping Mercury Electrode and by Direct Titration of Sulfited Dehydrated Cabbage, Carrots, and Potatoes

Commodity	Direct titration	Sulfur Dioxide
	P.p.m.	Mercury electrode P.p.m.
Cabbage	1104, 1070	1100, 1050, 1060
Carrots	489, 499	483, 465
Potatoes A	687, 653	675, 690
Potatoes B	2160, 2225	2140, 2150

Table VII. Recovery of Sulfite Added to Unsulfited Dehydrated Cabbage Suspensions

(Dropping mercury electrode data)		
$\text{SO}_2$ Added Mg.	$\text{SO}_2$ Found Mg.	Recovery %
0.486	0.465	95.7
0.486	0.485	99.8
0.486	0.475	97.7
0.972	0.930	95.7
0.972	0.920	94.6
0.972	0.925	95.2

recovery of sulfur dioxide added to cabbage and carrots is shown in curves 3 and 7, respectively, of Figure 2, which resemble curve 3 in Figure 1. Additional recovery data for cabbage are presented in Table VII. A typical curve showing the effect of acetone is included in Figure 2. The conditions represented by curves 4 and 5 differ only in that for the latter they include the addition of acetone. With acetone present, sulfited cabbage yields the same curve as the unsulfited cabbage and the supporting electrolyte. The use of acetone or other binding agent is unnecessary in the polarographic procedure. The curve included here to demonstrate the completeness of reaction between sulfurous acid and acetone.

In analyses of dehydrated vegetables 1 gram of the material was weighed into the electrolysis vessel and 48 ml. of water were added. Larger amounts of dried material may be used provided the suspensions produced are not too viscous to work with conveniently. The distilled water used had been swept free of oxygen by passing a vigorous current of nitrogen through it for 30 minutes. One-half milliliter of  $5 N$  sodium hydroxide was added, the suspension was allowed to stand 10 to 30 minutes, and 1.5 ml. of  $5 N$  hydrochloric acid were added. Measurements of the diffusion current were commenced immediately.

No oxygen wave has been noted during analysis of samples of sulfited dehydrated vegetables. This is to be expected considering the well-known ability of sulfur dioxide to remove oxygen quantitatively under alkaline conditions. Loss of sulfur dioxide is minimized by the use of oxygen-free water and avoidance of vigorous agitation which might incorporate oxygen. With material that has not been previously treated with sulfur dioxide it is of course necessary to remove dissolved oxygen by bubbling a stream of inert gas through the suspension.

Results obtained by the dropping mercury electrode as compared with the direct titration method are given for dehydrated cabbage, carrots, and potatoes in Table VI. Recovery of sulfur dioxide added to dehydrated cabbage is shown in Table VII.

## DISCUSSION

From the data presented, it is concluded that the direct titration method gives reproducible results and has an accuracy comparable with that of the distillation methods, especially in the range of 500 to 4000 p.p.m. of sulfur dioxide. It has the advantage of requiring less time than the distillation method and very little equipment. It can be mastered readily by an inexperienced analyst and should prove helpful for inspection and control laboratories and field stations. The results obtained by the direct titration and distillation methods are in agreement with those obtained with the dropping mercury electrode, which yields data specific for sulfur dioxide under the conditions used.



In addition, the direct titration procedure is capable of providing information not obtainable by the distillation methods. For example, omission of the alkali in the two titrations will yield the free sulfur dioxide, and the sulfur dioxide present in the bound form can then be estimated by determining the total described above. The determination will not be precise, because a measurable time will be needed to leach out the sulfur dioxide from the food particles, and the equilibrium in the suspension may have shifted appreciably from that in the dehydrated material.

The various methods yield results that show general agreement. Results by the Nichols-Reed method disagree with those by the other methods on cabbage and onions, when the total sulfite is low. On cabbage at higher sulfite levels, the results closely approximate those obtained by the other procedures. Fresh unsulfited cabbage may yield iodine-reducing distillates which, calculated as sulfur dioxide, are equivalent to 2000 to 3000 p.p.m. on the dry basis. Most of the natural reducing material is lost during dehydration; in control-dried cabbage, for example, analogous figures vary from 300 to 1000 p.p.m. This material does not affect the Monier-Williams or the di-

rect titration values, which are therefore preferred for these commodities.

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## Determination of Sodium in Potassium Hydroxide

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Sodium is separated from potassium by extraction of perchlorates with isopropanol and is precipitated from the alcoholic filtrate with magnesium uranyl acetate reagent containing a relatively high concentration of uranium. The method is rapid and economical, and requires no temperature control during precipitation. Recovery of sodium is 94% complete and accurate results are obtained by means of an empirical factor. The limit of uncertainty of the method under the best conditions was found to be  $\pm 0.009\%$  for a sample analyzing 0.082% sodium hydroxide; under routine laboratory conditions it was  $\pm 0.018\%$  for a sample analyzing 0.085% sodium hydroxide.

THE sodium content of potassium hydroxide has been determined in this laboratory for a number of years by separation of most of the potassium as the perchlorate, followed by precipitation of the sodium with magnesium uranyl acetate. Two procedures have been used: (1) separation of the potassium in aqueous solution followed by precipitation of the sodium with the Caley-Foulk reagent (8); (2) separation of the potassium in isopropanol solution followed by precipitation of the sodium with a reagent having a higher concentration of uranium than that recommended by Caley and Foulk.

The use of the reagent recommended by Caley and Foulk has a number of disadvantages. Precipitation is slow and requires mechanical stirring for a half hour for completion. A very large excess of dilute reagent is used. This increases the cost and exaggerates solubility effects. As a result of the latter, close temperature control and saturation of the reagent with sodium are required.

The Caley-Foulk reagent contains 35 times as much magnesium as uranium, expressed in terms of an equivalent quantity of sodium. It was found that the characteristics of the reagent could be improved by increasing the uranium concentration tenfold at the expense of a slight decrease in the concentration of the magnesium. Only a relatively small volume and a slight excess of this reagent, calculated on the basis of the uranium, are required. Temperature effects are negligible, owing to the low solubility of sodium in this reagent. Prolonged agitation is not required and precipitation is complete in a few minutes (in a few seconds in most cases).

This reagent has been used for the precipitation of sodium from both alcoholic and aqueous solutions and is equally satisfactory in both media. Relatively small amounts of potassium interfere and for this reason it is necessary to utilize an organic solvent for separating most of the potassium from the sodium prior to precipitation of the sodium. Of the solvents tested for this purpose, 99% isopropanol is the most satisfactory. Isopropanol is also the most satisfactory solvent tested for transferring and washing the precipitate. The precipitate is weighed after drying at 110° C.

#### REAGENTS

**MAGNESIUM URANYL ACETATE.** Dissolve 160 grams of uranyl acetate dihydrate, 180 grams of magnesium acetate tetrahydrate, and 45 grams of glacial acetic acid in 750 ml. of distilled water by heating to about 70° C. with stirring. Cool to 25° C., dilute to 1 liter, and filter before using. The specific gravity of this reagent should be  $1.169 \pm 0.005$  at 25°/15° C.

**ISOPROPANOL, 99%,** supplied by the Carbide and Carbon Chemicals Corporation as the anhydrous grade.

**PERCHLORIC ACID, 70 to 72%, C.P.**

#### PROCEDURE

Weigh a 1-gram portion of solid potassium hydroxide or 2 grams of a liquid sample into a 180-ml. tall-form beaker and dilute to about 10 ml. Add a drop of phenolphthalein and neutralize by the dropwise addition of 70% perchloric acid, adding 1.0 ml. of acid in excess of the neutral point. Evaporate carefully, to avoid spattering, on a hot plate until dense white fumes of perchloric acid appear and then fume 0.5 minute more. After a little experience the perchloric acid fumes can readily be distinguished from the less dense water vapor. The whole evaporation should be performed at such a temperature that spattering will not occur; 15 minutes is about the minimum time in which this can safely be accomplished.

After the evaporation is completed, cool the beaker and contents to room temperature in a stream of water, and extract the precipitate with successive 5-ml. portions of isopropanol. Caution should be exercised at this point to avoid the danger of adding organic matter to hot perchloric acid. The extraction can best be accomplished by adding the isopropanol from a small fine-tipped wash bottle up to a predetermined mark upon the side of the beaker. Stir the mixture thoroughly, breaking up any large lumps of solid material, and decant as much of the liquid and as little of the solid as possible through a Gooch crucible containing a disk of Whatman No. 40 filter paper. (What-



man No. 40 disks, 21 mm. in diameter, to fit No. 3 Coors Gooch crucibles, may be obtained from H. Reeve Angel and Co., New York.) Repeat this operation three more times, transferring the precipitate to the crucible with the last 5-ml. portion. Rinse the beaker into the crucible once with a fine jet of isopropanol and wash down the sides of the crucible similarly, using a minimum of alcohol. Transfer the contents of the suction flask to a dry 180-ml. tall-form beaker and rinse the flask with a minimum of isopropanol. The volume of the beaker contents should be about 25 ml. at this point.

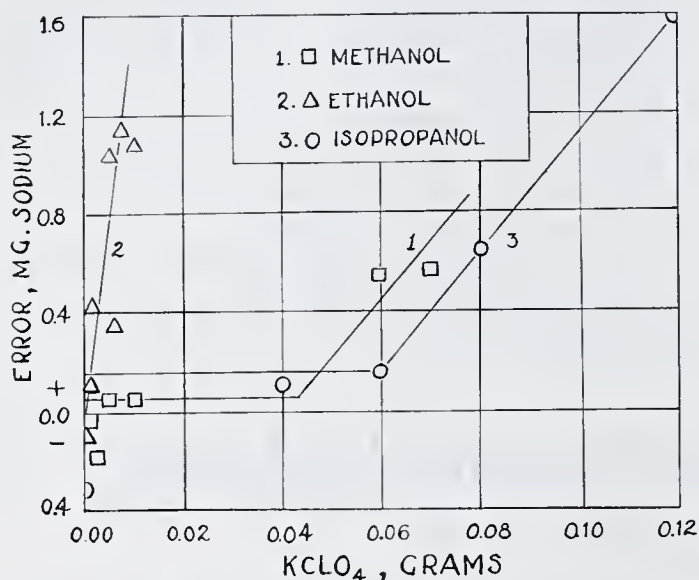


Figure 1. Effect of Potassium in Alcoholic Solution

■ Precipitate the sodium by adding 10 ml. of the magnesium uranyl acetate reagent slowly to the alcoholic solution with swirling. Continue to swirl for 20 seconds. Allow the precipitate to stand for 10 minutes or longer and filter through a tared Gooch crucible containing a disk of Whatman No. 40 filter paper. Transfer the precipitate and rinse the beaker with four or five small portions of isopropanol. Wash down the sides of the crucible with isopropanol, using no more than 25 ml. for the entire transfer and washing operation. Remove the crucible carefully, wipe off the outside, dry 5 minutes at 105° to 110° C., cool, and weigh. Calculate per cent sodium hydroxide as follows:

$$\frac{\text{Grams of ppt.} \times 0.0261 \times 100}{\text{Grams of sample} \times 0.94} = \% \text{ NaOH}$$

#### EXPERIMENTAL

The reagent recommended by Caley and Foulk (3) contains 42.5 grams per liter of uranyl acetate dihydrate, 250 grams per liter of magnesium acetate tetrahydrate, and 60 grams per liter of glacial acetic acid. From 1 to 5 ml. of solution containing up to 50 mg. of sodium are mixed with 100 to 500 ml. of the reagent and stirred vigorously for 30 to 45 minutes at 20° C. Because of the appreciable solubility of sodium in this reagent and the large volume used, it is necessary to saturate the reagent prior to use and store at a temperature above 20° C. The uranium content of the reagent is equivalent to 0.77 mg. of sodium per ml., while the magnesium content is equivalent to 27 mg. per ml. It seemed desirable, therefore, to make the uranium and magnesium content more nearly equivalent. It was found that solutions could be prepared which contained 180 grams per liter of each salt, but that uranyl acetate precipitated from this solution upon stirring at 20° C. For this reason, 160 grams per liter of uranyl acetate tetrahydrate were chosen as the maximum practical concentration.

The concentration of magnesium acetate tetrahydrate must be maintained somewhat below 250 grams per liter in order to permit the required amount of uranyl acetate to dissolve. Some of the experimental work was performed with a reagent containing 160 grams per liter of the magnesium salt, but a batch prepared from a reagent labeled  $\text{Mg}(\text{C}_2\text{H}_3\text{O}_2)_2 \cdot x\text{H}_2\text{O}$  resulted in low recoveries of sodium and apparently contained only about 130

grams per liter of the tetrahydrate. This observation suggested the approximate lower limit for the magnesium acetate concentration and 180 grams per liter were adopted as providing a desirable margin of safety. The use of 10 ml. of this concentrated reagent in place of 100 ml. of the Caley-Foulk reagent reduces the cost per determination from about \$0.17 to less than \$0.04.

In view of the extremely high potassium-sodium ratio of caustic potash, the possibility of determining sodium without prior separation of the potassium appeared remote. However, the advantages of such a procedure were so great that it was considered advisable to investigate this possibility. Conditions were found which prevented the precipitation of potassium in the absence of sodium, but the same conditions resulted in large positive errors in the recovery of added sodium. Moreover, when the sodium magnesium uranyl acetate was dissolved and precipitated under the same conditions as before, the weight of the second precipitate bore no relationship to the amount of sodium present. Thus, the direct determination of sodium with caustic potash by either one or two precipitations as the triacetate appears to be unsatisfactory.

Having eliminated the direct determination of sodium in the presence of potassium, the procedure resolved itself into two parts: (1) separation of a large portion of the potassium as perchlorate; (2) precipitation of the sodium. The simplest procedure for separation of the potassium would involve acidifying the sample with perchloric acid, removing the precipitated potassium perchlorate by filtration of the cold aqueous solution, and precipitating the sodium in the filtrate. To determine the effect of the potassium remaining in this filtrate varying amounts of potassium perchlorate were added to portions of sodium perchlorate, equivalent to 4.0 mg. of sodium. The volume of each solution was adjusted to 5 ml. and the sodium precipitated with 10 ml. of magnesium uranyl acetate. The data obtained indicated that potassium perchlorate in excess of 70 mg. would probably interfere under these conditions. The solubility of potassium perchlorate in water is such that it appeared impractical to reduce the concentration below this level.

Table I. Solubilities of Sodium and Potassium Perchlorates

Solvent	NaClO <sub>4</sub>	KClO <sub>4</sub>	$\frac{\text{NaClO}_4}{\text{KClO}_4}$
	G./25 ml.	G./25 ml.	Ratio $\times 10$
Methanol	7.81	0.0207	3.8
Ethanol	3.02	0.0025	12.1
Isopropanol	0.62	0.0015	4.1
Isopropanol, 91%	0.81	0.0064	1.3

Barber and Kolthoff (2) separated potassium from sodium with ammonium perchlorate in 72% ethanol and precipitated sodium from the aqueous solution after evaporating the alcohol. Willard and Diehl (7) list the solubilities of sodium and potassium perchlorates in several alcohols and mixed solvents. The high sodium perchlorate-potassium perchlorate solubility ratio indicated the possibility of separating the sodium from the potassium by extraction, prior to the sodium determination. In pursuance of this, the approximate solubilities of sodium and potassium perchlorates in several solvents were determined. To avoid separation of the solvents, the study was limited to alcohols which were miscible with the magnesium uranyl acetate reagent. The alcohols were technical materials and were used without further purification. They are described by the supplier as follows: methanol, not less than 99.85% by weight; ethanol, absolute; isopropanol, approximately 91% by volume; and isopropanol, not less than 99.4% by volume. The following procedure was used.

To 100-ml. portions of the alcohols was added excess of finely pulverized salt. After heating to 50° C. to hasten solution the mixtures were agitated vigorously for 2 hours at 25° C. The excess salt was allowed to settle, the supernatant liquid was filtered, 25-ml. portions of the filtrate were evaporated to dryness in tared weighing bottles, and the residue was weighed.



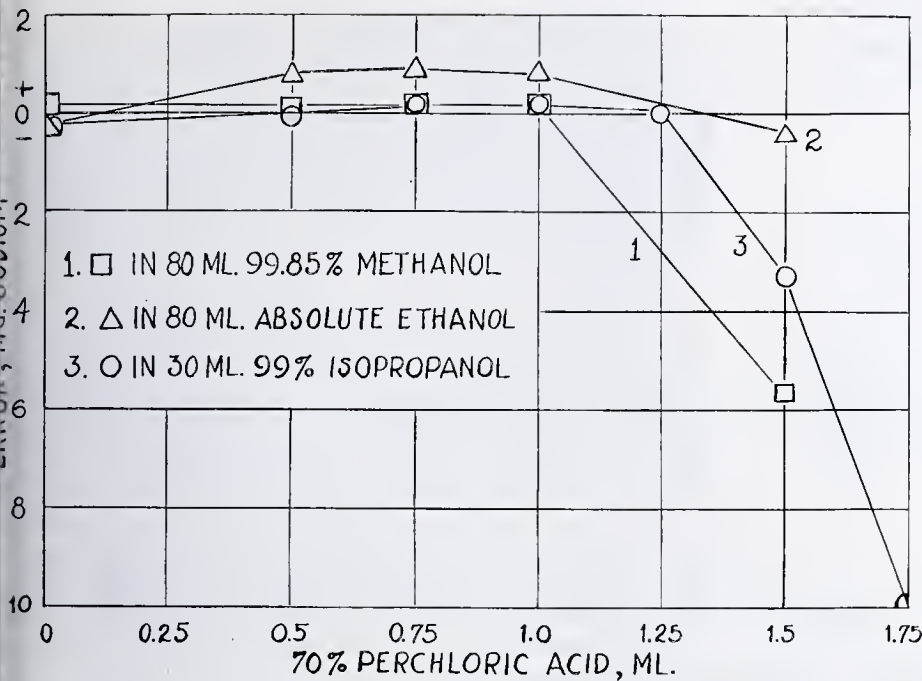


Figure 2. Effect of Perchloric Acid on Recovery of Sodium

The data obtained (Table I) indicate that ethanol gives by far the most favorable ratio of solubility of sodium perchlorate to potassium perchlorate, while methanol and 99% isopropanol give about equal ratios. Dilution of isopropanol to 91% substantially increased the ratio. This suggested the desirability of making the separation in an essentially anhydrous medium.

The effect of potassium in alcoholic solutions was determined by precipitating 10 mg. (13.3 mg. were used in some tests) of sodium from 25 ml. of the alcohol. To simulate actual working conditions 0.75 ml. of 70% perchloric acid was added to each solution. The data presented in Figure 1 show that ethanol is entirely unsatisfactory as a solvent for the precipitation. If ethanol is used as the solvent, the amount of potassium perchlorate which may be present without causing appreciable interference is about twice the solubility of potassium perchlorate in ethanol, while the corresponding value for 99% isopropanol is forty-fold. It follows that isopropanol is the most satisfactory solvent for the separation and precipitation of the sodium.

Since the complete removal of perchloric acid was impractical, the maximum amount which could be tolerated was determined. The data obtained, shown graphically in Figure 2, indicate that amounts up to 1.0 ml., and probably more, do not interfere in any of the three alcohols tested. Positive errors were obtained at intermediate acid concentrations when using ethanol as the solvent. While the cause of this anomalous behavior was not determined, it may be due to variations in the degree of solvation.

A 30-minute stirring period was utilized in all the experimental work described above to ensure complete precipitation of the sodium magnesium uranyl acetate. To determine the possibility of shortening the stirring period, 3.0 mg. of sodium, 2.0 mg. of potassium, and 0.5 ml. of 70% perchloric acid were dissolved in 10 ml. of 99% isopropanol; 10 ml. of magnesium uranyl acetate were added and the solutions were agitated for varying periods. The data obtained are presented in Table II and indicate that precipitation is complete after 10 seconds of swirling. A 20-second swirling period was adopted to provide a safety factor. Subsequent experience with the routine application of the method indicated that better precision was obtained by allowing the precipitate to stand 10 minutes, after swirling and before filtering.

The customary wash solution used for sodium magnesium uranyl acetate is 95% ethanol, shaken and maintained in contact with an excess of the precipitate. This and other solvents were tested as wash solutions by determining the solubility losses dur-

ing washing. Five 0.65-gram portions of the triple acetate precipitate, corresponding to about 10 mg. of sodium, were washed in Gooch crucibles with successive 25-ml. portions of the various solutions. Table III shows that methanol is unsatisfactory as a wash solution; 95% ethanol in contact with excess precipitate and 91% isopropanol are about equally satisfactory; 99% isopropanol is somewhat superior to the last two; and the solubility of the precipitate in acetone is negligible. However, acetone was found to precipitate the reagent, leaving considerable residue in the beaker. Thus, although acetone is excellent for washing the precipitate prior to drying, it is not satisfactory for transferring or washing the precipitate free of excess reagent. For this reason, 99% isopropanol was selected as the most satisfactory wash solution considered. The precipitate may be dried at 105° to 110° C. or by aspirating air through it. If the latter method is used, the precipitate should be washed with a small volume of acetone to take advantage of its higher vapor pressure and to reduce the time required to

evaporate the solvent completely.

Caley and Foulk (3) found that the precipitate formed in aqueous solutions is solvated with 6.5 molecules of water. Schoorl (6) and Caley and Rogers (4) found independently that magnesium uranyl acetate which has been precipitated from aqueous-ethanolic solutions is solvated with ethanol as well as water. The authors' experience with the precipitate from aqueous solutions showed that it readily attained constant weight at temperatures up to 120° C., lost weight slowly at 150° C., rapidly at 180° C., and quickly blackened at 200° C. On this basis a portion of the precipitate from isopropanol solution was dried at 145° to 150° C. for an extended period. The data, plotted in Figure 3, show a marked change in the rate of loss in weight after 6 hours, the continued loss in weight being attributed to decomposition of the molecule. Extrapolation of the curve indicates a rapid loss of 9.4 or 9.5%, depending upon whether decomposition is assumed to begin immediately upon heating or not until desolvation is complete. The isopropanol content of portions of

Table II. Effect of Time of Agitation  
(3.0 mg. of Na added)

Agitation	Sodium Found <sup>a</sup> Mg.	Error Mg.
Stirred mechanically 30 min.	3.02	+0.02
Stirred mechanically 15 min.	3.04	+0.04
Stirred mechanically 5 min.	3.01	-0.01
Swirled manually 60 sec.	3.07	+0.07
Swirled manually 10 sec.	3.09	+0.09
Swirled manually 5 sec.	2.89	-0.11
Swirled manually 2 sec.	2.85	-0.15

<sup>a</sup> After subtracting blank of 0.11 mg.

Table III. Solvent Action of Wash Solutions on Sodium Magnesium Uranyl Acetate

Washing	Loss of Precipitate				
	Methanol, 99.85%	Ethanol, 95% <sup>a</sup>	Isopropanol, 91%	Isopropanol, 99%	Acetone
	Gram/25 ml.				
1	0.076	0.004	0.006	0.001	0.000
2	0.089	0.003	0.003	0.001	0.000
3	0.096	0.001	0.001	0.001	0.000
4	...	0.003	0.002	0.001	0.001
5	...	...	0.001	...	...
Av.	0.087	0.0028	0.0033	0.0010	0.0003

<sup>a</sup> Maintained in contact with sodium magnesium uranyl acetate precipitate.



Table IV. Recovery from 50% Potassium Hydroxide

Added %	NaOH		Recovery %
	Found <sup>a</sup> %	Error %	
0.000	0.000	0.000	..
0.000	0.000	0.000	..
0.002	0.003	+0.001	..
0.010	0.011	+0.001	..
0.050	0.047	-0.003	94
0.100	0.093	-0.007	93
0.498	0.478	-0.020	96
0.995	0.919	-0.076	92
1.99	1.85	-0.14	93

<sup>a</sup> After subtraction of blank of 0.003%.

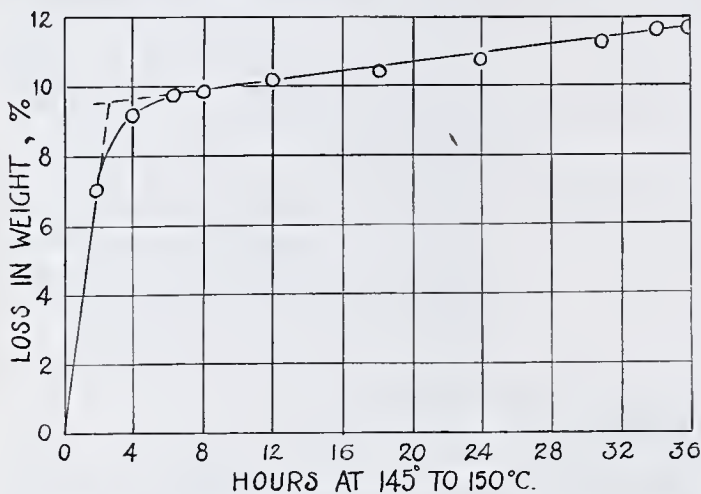


Figure 3. Drying of Sodium Magnesium Uranyl Acetate  
Loss in weight at 145° to 150° C.

the precipitate was determined by oxidation with dichromate in sulfuric acid solution, followed by titration of the excess dichromate with ferrous sulfate using diphenylamine sulfonic acid as the indicator. The recovery of known amounts of isopropanol was 95% complete under the conditions used. The isopropanol content found on duplicate portions of the precipitate was 1.04 and 1.06%. By difference, the water content is 8.4%. These data correspond to a precipitate containing 7 molecules of water and 0.3 molecule of isopropanol and having a molecular weight of 1534. Thus, the theoretical factor for converting to sodium is 0.0150.

To determine the volume of isopropanol required to extract the sodium perchlorate from the potassium perchlorate, synthetic samples containing added sodium equivalent to 0.87% of sodium hydroxide were carried through the procedure except that varying volumes of isopropanol were used. Extraction with 10 ml. of isopropanol resulted in the recovery of 0.86%, and 0.88% was recovered when 30 ml. was used. These data indicate that the sodium is readily extracted and that 20 to 25 ml. should be adequate for routine use.

To determine the accuracy of the procedure, a sample of low-sodium potassium hydroxide was prepared by a method similar to that of Richards and Mueller as described by Archibald (1). c.p. potassium oxalate was recrystallized from water and a hot saturated solution prepared. This was placed in a large porcelain evaporating dish and electrolyzed, using a mercury pool as cathode and a platinum disk as anode. The contents were cooled by immersing the dish in ice water. When the mercury began to solidify, the supernatant liquid was discarded and the amalgam washed three times with intermittent electrolysis until the wash water was free of oxalate ion. Distilled water was added to the amalgam and the current reversed to hasten the formation of the potassium hydroxide. The aqueous solution was then evaporated in a porcelain casserole to 48% potassium hydroxide and stored in a hard-rubber bottle to avoid sodium contamination.

The purified potassium hydroxide was diluted so that 10 ml. were equivalent to a 2-gram sample of 45% potassium hydroxide. To 10-ml. portions of this solution were added varying portions of standard 0.1 N sodium hydroxide and the solutions were analyzed. Duplicate portions containing no added sodium were found to contain 0.003% sodium hydroxide. This was considered to be a blank determination and was subtracted from the actual analyses shown in Table IV.

These data show preponderantly negative errors with fairly consistent recoveries of about 94% of the added sodium. This indicates that the method is best applied to the determination of small amounts of sodium where the absolute errors observed are less serious. However, by means of an empirical factor for converting the weight of the precipitate to per cent sodium hydroxide the accuracy can be made equal to the precision of the method which is shown to be very good.

The precision of this procedure was determined under the best conditions and under routine conditions as described by Morawitz (5). Assuming random distribution and applicability of the normal law integral, 68.3% of all analyses would be expected to lie within the range of the average of a group of analyses plus or minus the standard deviation for an infinite group. Similarly, 99.7% of all analyses should lie within the range of the average plus or minus three times the standard deviation for an infinite group. The latter range has been termed the limit of uncertainty, *LU* (5). The precision under the best conditions, *LU*<sub>1</sub>, was determined by the analyses of 10 portions of a homogeneous sample (Table V). The *LU*<sub>1</sub> of ±0.009% in a sample analyzing 0.082% sodium hydroxide is considered satisfactory. The precision

Table V. Precision of Method under Best Conditions, *LU*<sub>1</sub>

Analysis No.	NaOH, %	Deviation from Average, %
1	0.083	+0.001
2	0.083	+0.001
3	0.086	+0.004
4	0.075	-0.007
5	0.083	+0.001
6	0.083	+0.001
7	0.085	+0.003
8	0.081	-0.001
9	0.082	0.000
10	0.082	0.000
Av.		0.0823
Standard deviation of group		±0.0028
Standard deviation of infinite group <i>LU</i> <sub>1</sub>		±0.0030 ±0.009

Table VI. Precision of Method under Routine Conditions, *LU*<sub>1</sub>

Analysis No.	Date	NaOH, %	Deviation from Average, %
1	May, 1941	0.088	+0.003
2	May, 1941	0.087	+0.002
3	June, 1941	0.086	+0.001
4	June, 1941	0.087	+0.002
5	July, 1941	0.077	-0.008
6	July, 1941	0.087	+0.002
7	August, 1941	0.074	-0.011
8	August, 1941	0.099	+0.014
9	September, 1941	0.085	0.000
10	September, 1941	0.085	0.000
11	October, 1941	0.094	+0.009
12	October, 1941	0.090	+0.005
13	November, 1941	0.084	-0.001
14	November, 1941	0.082	-0.003
15	December, 1941	0.076	-0.009
16	December, 1941	0.085	0.000
17	January, 1942	0.076	-0.009
18	January, 1942	0.070	-0.015
19	January, 1942	0.086	+0.001
20	February, 1942	0.084	-0.001
21	February, 1942	0.086	+0.001
22	March, 1942	0.094	+0.009
23	March, 1942	0.089	+0.004
24	April, 1942	0.087	+0.002
25	April, 1942	0.090	+0.005
26	May, 1942	0.088	+0.003
27	May, 1942	0.084	-0.001
28	June, 1942	0.088	+0.003
29	June, 1942	0.090	+0.005
30	July, 1942	0.086	+0.001
31	July, 1942	0.085	0.000
32	August, 1942	0.075	-0.010
33	August, 1942	0.075	-0.010
34	September, 1942	0.090	+0.005
35	September, 1942	0.087	+0.002
36	October, 1942	0.087	+0.002
37	October, 1942	0.089	+0.004
38	November, 1942	0.090	+0.005
39	November, 1942	0.088	+0.003
40	December, 1942	0.088	+0.003
41	December, 1942	0.085	0.000
Av.			0.085
Standard deviation of group			±0.0058
Standard deviation of infinite group <i>LU</i> <sub>2</sub>			±0.0059 ±0.018



tion under routine conditions,  $LU_2$ , was determined from analyses on the same sample over a period of 20 months. These data, representing 41 analyses by seven different analysts and shown in Table VI, indicate an  $LU_2$  of  $\pm 0.018\%$  and an average of  $0.085\%$  sodium hydroxide. This  $LU_2$  represents a rigorous test of the analytical method and the  $LU_2$ - $LU_1$  ratio of 2 to 1 is considered normal and as indicating the effect of personal, seasonal, and other variations.

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## Refractive Index—Dry Substance Tables for Starch Conversion Products

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The refractive index of starch hydrolyzate sirups has been found to measure their dry substance content, provided the ash and dextrose equivalent are also known. All commercial corn sirups are marketed with essentially the same ash content, largely a function of the dextrose equivalent. Thus with the dextrose equivalent known, refractive indices are readily defined in terms of dry substance. Fortunately the correction for dextrose equivalent is small and

linear. Likewise, the temperature correction, above  $14\%$  dry substance, is substantially linear. The precision of this method of determining dry substance is essentially the precision of a four-place refractometer. Tables are presented covering refractive index—dry substance for the usual commercial products from 0 to  $90\%$  dry substance and refractive index—commercial Baumé, since all corn sirup is marketed on a commercial Baumé basis.

STARCH conversion products for the purposes of this paper are defined as corn sirups and Nos. "70" and "80" corn sirups, made by the simple hydrolysis of starch by acid. This definition automatically excludes dual conversion sirups—those followed by enzymic hydrolysis and interconversion sirups made by chemical treatment on an acid hydrolyzate. Of the products mentioned, corn sirup is the only one which reaches the market as a sirup. The "70" and "80" sugars reach the consumer in the form of crystallized chips or billets. However, these products are sirups in the factory, and since the moisture at shipping is essentially the moisture at pouring, refractive index tables for these products are valuable for manufacturing control. Corn sirup is the thick, viscous, substantially colorless sirup obtained from the incomplete hydrolysis of starch. It is sold on the basis of Baumé and dextrose equivalent and in the trade is often referred to as corn sirup unmixed or, more simply, U. The Baumé (commercial) will run from  $42^\circ$  to  $47^\circ$ , the greater part being  $43^\circ$ . Dextrose equivalent (D.E.) is defined within the industry as the percentage of reducing sugars calculated as dextrose, expressed on a dry substance basis. Under this general classification, commercial sirup falls roughly into four groups: brewers' body sirup, 25 to 35; standard confectioners' sirups, 40 to 45; extra sweet sirups, 50 to 57; and dual conversion sirup (acid plus enzyme), 60 to 70 D.E. The names of the first three groups are general, in that there is considerable interchange in the use of these sirups.

The employment of Baumé as the basis for the sale of corn sirup follows a natural custom for liquids or sirups. The hydrometer, which has always been used, has been standardized at  $60^\circ$  C. ( $60^\circ$  F.). However, a Baumé test on commercial sirups at this temperature is impossible because they are too viscous. To eliminate this difficulty, an arbitrary method of

Baumé determination was adopted years ago and is still in use. The sirup under test is heated to  $140^\circ$  F. ( $60^\circ$  C.) and Baumé reading obtained with a hydrometer standardized at  $60^\circ$  F. To the observed reading at  $140^\circ$  F. is added  $1.00^\circ$  Bé. which was originally purported to reduce the reading to  $37.78^\circ$  C. ( $100^\circ$  F.). This method for the sale of corn sirup is designated as commercial Baumé and expressed as commercial Baumé = Baumé ( $140^\circ/60^\circ$  F.) +  $1.00^\circ$  Bé. Although this procedure simplifies the test, it introduces other difficulties (5), so that the Baumé test requires considerable skill in order to obtain results of a fine degree of precision. Since the Baumé scale is used as a measure of the dissolved solids or dry substance in a sirup, this relationship is of particular interest to manufacturers and users. Tables giving this relationship have been presented (5). However, determinations of Baumé as outlined above and of dry substances are laborious and time-consuming. A more rapid test for Baumé or dry substance has been desired for a long time. Refractive index has been proposed and the Abbe refractometer has been suggested for its ease of manipulation and speed of operation.

#### HISTORICAL

A few tables have been published on the refractive index—dry substance relationship for starch conversion products. Most of these lack definition through a failure to define adequately the dextrose equivalent of the product (9). More recently a table has been published in Germany (6) but only the abstract has reached this country. The fault with such tables as have been prepared rests upon (1) inadequate methods for determination of dry substance, (2) failure to define the dextrose equivalent which in turn depends upon the dry substance, and (3) failure to recognize that the refractive index for a given dry substance is also a function of the dextrose equivalent.

Adequate methods for the determination of dry substance in starch conversion products (3, 4) have enabled more accurate

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data to be obtained for the dextrose equivalent. Thus a refractive index table became a matter of the collection of sufficient data. These data have been compiled and in use in this laboratory for six years. The original data have been extended to a complete range of starch conversion products and a wide range of temperatures. Although this would at first appear to necessitate extended tables, considerable simplification was possible.

#### MATERIALS

The materials used were commercial products and typical of each class:

	D.E.	Ash	Crude Protein, Dry Basis
Brewers' body sirup	32.8	0.25	0.04
Confectioners' corn sirup	42.0	0.28	0.04
High conversion corn sirup (extra sweet corn sirup)	55.0	0.30	0.04
"70" sugar	82.0	0.41	0.07
"70" sugar	89.0	0.61	0.07
"80" sugar	90.7	1.22	0.08

With the exception of the "80" sugar, the ash content of the others is essentially proportional to the dextrose equivalent, which proved advantageous for plotting refractive index *vs.* dextrose equivalent. Of the materials mentioned above, those of 42.0, 55.0, 89.0, and 90.7 D.E. were either diluted or concentrated for a series of sirups covering the complete range of dry substance from 0 to 90%. The method used for this procedure has been published (5). The products of 32.8 and 82.0 D.E. were used in the concentrated ranges of 70 to 90%-dry substance. In addition, the refractive index-dry substance-Baumé relationship was determined for specific Baumés on innumerable samples of finished factory products and on similar samples manufactured by competitors during the six years the tables have been in use.

#### METHODS OF ANALYSIS

**MOISTURE.** The determination of moisture in corn sirup and corn sugar has been previously described (3, 4, 5). The methods used were as follows:

**Corn Sirup.** Filter-Cel method (diatomaceous silica, Johns-Manville Hy-Flo), vacuum oven at 100° C.; alternative methods, toluene distillation and benzene distillation.

**Corn Sugar.** For 80 to 92 D.E., Filter-Cel method, vacuum oven at 80° C.; alternative, benzene distillation.

**BAUMÉ BY HYDROMETER.** Baumé determination has been previously described (5). The Corn Industries Research Foundation's official referee hydrometers manufactured by Wm. Hergesell & Sons, New York, N. Y., were used. The sirup under test was poured into the cylinder (37.5 × 5.6 cm., 15 × 2.25 inch, Pyrex, without lip). The cylinder was sealed with dual stoppers, the bottom stopper being placed within 1.25 cm. (0.5 inch) of the sirup surface and the top stopper closed the cylinder. The cylinder was placed in the water bath (140° F.), so that water extended to within 2.5 cm. (1 inch) of the top. At the same time, distilled water was added to a second cylinder, the test hydrometer inserted in it, and the cylinder placed in the water bath. After the sirup was freed of air, the cylinder was placed on a higher shelf, so that the surface of the sirup extended about 1.25 cm. (0.5 inch) above the water after the hydrometer was immersed. The dual stoppers were removed from the cylinder. The test hydrometer was removed, dried, and immersed in the sirup. The hydrometer was read in approximately 10 minutes. To the reading obtained was added the correction necessary from a previous comparison of the hydrometer with one certified by the National Bureau of Standards.

**BAUMÉ BY PYCNOMETER.** A pycnometer in the form of a sphere with standard taper joints of Pyrex was designed and used. The apparent specific gravities by pycnometers on various concentrations of corn sirup, employing the correction whereby the apparent specific gravity of pycnometers could be translated to hydrometer readings, were compared with actual readings by hydrometers in the same sirup and found to agree within 0.01° B.

**REFRACTIVE INDEX. Apparatus.** Water bath, 120-liter (32-gallon), lagged, equipped with stirrer and metastatic mercury regulator with sensitivity of 0.02° C. Water was transferred from bath to refractometer by means of a pump, with capacity of 37.85 liters (10 gallons) per minute.

Light source, 60-watt frosted Mazda bulb.

Zeiss sugar refractometer, new model with rotating compensator, standardized at 20.0° C., Serial No. 54,128. The new model Zeiss sugar refractometer consists of a circular casing, con-

taining the mechanism, mounted on an upright stand. The height of stand is such that the ocular is at a convenient height for reading when the instrument is placed on a table. The fixed prism of the double prism is anchored in the casing and is placed that the inner surface of the prisms are placed horizontally side by side, when the hinged prism is folded back for cleaning or recharging. The telescope, carried on a stout radial arm, almost wholly enclosed, only the ocular protruding.

In making observations, the eye looks horizontally into the ocular. The image in the ocular presents the following appearance: In place of the usual cross lines, the field of view shows a linear mark, on the right of which is the percentage scale ranging from 0 to 50% in 0.2% and from 50 to 95% in 0.1%. On the left is a scale graduated in refractive indices, ranging from 1.33 to 1.540, graduated to the third decimal, the fourth decimal being estimated. The fact that reading is taken in the field of view ensures a considerable saving of time. The rotating compensator furnishes a means of rendering the boundary line between the bright and dark portions of the field, sharp and free of color fringes where white light illumination is employed and in addition renders the colorless boundary line exactly coincident in position with the sodium line—that is, the reading obtained with white light furnishes the refractive index,  $n_D$ , for sodium light.

Although any suitable refractometer may be used for making dry substance-Baumé determinations of starch conversion products, the authors have preferred the Zeiss sugar type because of the ease of cleaning and charging of samples. The instrument is rugged and has been used in many factories as a control instrument.

The range of the scale extends from  $n_D$  1.300 to  $n_D$  1.540, while the percentage readings range from 0 to 95%. The percentage scale is divided into fifths per cent from 0 to 50% and into tenths from 50% upwards. The refractive index scale reads to the third decimal direct, while the fourth decimal is estimated.

The constancy and precision of the refractometer were checked periodically. Distilled water and the test block supplied by the manufacturer for this particular instrument were employed at 20.0° C. For temperatures above and below 20° C., distilled water was used and the refractive index thus obtained compared to the value given in the International Critical Tables. During the past six years, the refractometer was found to check and in any case did not deviate more than one in the fourth place from these known refractive indices, which was deemed within the tolerance of experimental procedure.

**Temperature.** Refractive index values were obtained at 18.0°, 20.0°, 25.0°, 33.0°, 37.0°, 45.0°, and 50.0° C. on the same sample used for dry substance, Baumé, and specific gravity. Same handling has been described in detail in previous papers (3, 4).

The question of what refractometer temperature to use as a standard for the heavy commercial corn sirups (Baumé 40°) was given considerable attention. The temperature chosen was 45° C. based on the following considerations:

1. Speed in transfer and closing of the prisms is essential. The thick viscous corn sirups present difficulties. However, their viscosities decrease sharply with increasing temperature (2) and the proportional decrease is very small above 43.33° (110° F.).

2. Most corn products and candy factory laboratories come warm in the summertime and those in the midwest often attain temperatures of 100° F. or more. The relative humidity is also high. If the refractometer temperature is less than room temperature, there is a tendency for the prisms to fog. The condensed moisture hinders the reading and also effects a distortion of the sample (7, 8).

3. The refractometer is already in use in many candy factories as an instrument to measure the degree of cooking of starch jelly and the temperature used is generally 45° C. Therefore 45° C. enables such plants to measure the refractive index of corn sirup with existing temperature control (1).

Numerous studies were made to determine if any change took place in the sample when maintained at 45° C. The method usually employed was to introduce a sample at a low temperature and obtain a series of readings. The temperature was then elevated to 45° or 50° C. and held there for periods up to several hours while readings were taken at intervals. The bath was cooled down to the original temperature and another series of index readings was taken at intervals. The results at the low temperature were found to be constant and the readings at the high temperature before and after heating checked precisely. Hence it was concluded that the use of the refractometer at 45° C. resulted in no loss of precision if the transfer of the sample to the refractometer and the closing of the prisms were carried out with dispatch.

**PROCEDURE.** The method of applying the sirups to the refractometer is important and practice is the best direct



Table I. Dry Substance-Refractive Index

Dry substance	20.00° C.				45.00° C.			
	Dextrose Equivalent and Ash				Dextrose Equivalent and Ash			
	42.00 0.28%	55.00 0.30%	89.00 0.61%	90.7 1.22%	42.00 0.28%	55.00 0.30%	89.00 0.61%	90.7 1.22%
0.00	1.3330	1.3330	1.3330	1.3330	1.3298	1.3298	1.3298	1.3298
1.00	1.3344	1.3344	1.3344	1.3344	1.3312	1.3312	1.3312	1.3312
2.00	1.3359	1.3359	1.3359	1.3359	1.3327	1.3327	1.3326	1.3326
3.00	1.3374	1.3374	1.3373	1.3374	1.3341	1.3341	1.3340	1.3340
4.00	1.3389	1.3389	1.3388	1.3388	1.3356	1.3356	1.3355	1.3355
5.00	1.3404	1.3404	1.3403	1.3403	1.3371	1.3371	1.3369	1.3369
6.00	1.3419	1.3419	1.3418	1.3418	1.3386	1.3386	1.3384	1.3384
7.00	1.3435	1.3435	1.3433	1.3433	1.3401	1.3401	1.3399	1.3399
8.00	1.3450	1.3450	1.3448	1.3448	1.3416	1.3416	1.3414	1.3414
9.00	1.3466	1.3466	1.3463	1.3464	1.3432	1.3431	1.3429	1.3429
10.00	1.3482	1.3482	1.3479	1.3479	1.3447	1.3447	1.3444	1.3444
11.00	1.3498	1.3497	1.3494	1.3495	1.3463	1.3462	1.3459	1.3459
12.00	1.3514	1.3513	1.3510	1.3510	1.3479	1.3478	1.3475	1.3475
13.00	1.3531	1.3530	1.3526	1.3526	1.3495	1.3494	1.3490	1.3490
14.00	1.3547	1.3546	1.3542	1.3542	1.3511	1.3510	1.3506	1.3506
15.00	1.3563	1.3562	1.3558	1.3558	1.3527	1.3526	1.3521	1.3521
16.00	1.3580	1.3579	1.3574	1.3574	1.3544	1.3542	1.3537	1.3537
17.00	1.3597	1.3595	1.3590	1.3590	1.3560	1.3558	1.3553	1.3553
18.00	1.3614	1.3612	1.3606	1.3607	1.3576	1.3575	1.3569	1.3569
19.00	1.3632	1.3629	1.3623	1.3623	1.3593	1.3592	1.3585	1.3585
20.00	1.3649	1.3646	1.3640	1.3640	1.3610	1.3608	1.3602	1.3602
21.00	1.3666	1.3663	1.3656	1.3656	1.3627	1.3625	1.3618	1.3618
22.00	1.3684	1.3681	1.3673	1.3673	1.3645	1.3642	1.3635	1.3635
23.00	1.3701	1.3698	1.3690	1.3690	1.3662	1.3659	1.3652	1.3652
24.00	1.3719	1.3716	1.3707	1.3707	1.3680	1.3677	1.3669	1.3668
25.00	1.3737	1.3733	1.3725	1.3725	1.3698	1.3694	1.3686	1.3685
26.00	1.3755	1.3751	1.3742	1.3742	1.3715	1.3712	1.3703	1.3702
27.00	1.3774	1.3769	1.3759	1.3759	1.3733	1.3729	1.3720	1.3719
28.00	1.3792	1.3788	1.3777	1.3777	1.3751	1.3747	1.3737	1.3737
29.00	1.3811	1.3806	1.3794	1.3794	1.3770	1.3765	1.3754	1.3755
30.00	1.3829	1.3824	1.3812	1.3812	1.3788	1.3783	1.3772	1.3773
31.00	1.3848	1.3843	1.3830	1.3830	1.3806	1.3802	1.3789	1.3790
32.00	1.3867	1.3862	1.3848	1.3848	1.3825	1.3820	1.3807	1.3808
33.00	1.3886	1.3880	1.3866	1.3866	1.3844	1.3839	1.3826	1.3826
34.00	1.3906	1.3899	1.3885	1.3885	1.3863	1.3857	1.3844	1.3844
35.00	1.3925	1.3919	1.3903	1.3903	1.3882	1.3876	1.3862	1.3863
36.00	1.3945	1.3938	1.3922	1.3921	1.3901	1.3895	1.3880	1.3881
37.00	1.3964	1.3957	1.3941	1.3940	1.3921	1.3914	1.3899	1.3899
38.00	1.3984	1.3977	1.3959	1.3959	1.3940	1.3934	1.3917	1.3918
39.00	1.4004	1.3997	1.3978	1.3978	1.3960	1.3953	1.3936	1.3937
40.00	1.4024	1.4016	1.3997	1.3997	1.3980	1.3973	1.3955	1.3955
41.00	1.4044	1.4036	1.4016	1.4016	1.4000	1.3993	1.3974	1.3974
42.00	1.4065	1.4057	1.4036	1.4035	1.4020	1.4012	1.3993	1.3993
43.00	1.4086	1.4077	1.4055	1.4055	1.4041	1.4032	1.4012	1.4011
44.00	1.4107	1.4097	1.4074	1.4074	1.4061	1.4053	1.4031	1.4032
45.00	1.4128	1.4118	1.4094	1.4094	1.4082	1.4073	1.4051	1.4051
46.00	1.4149	1.4139	1.4114	1.4113	1.4103	1.4093	1.4071	1.4071
47.00	1.4170	1.4160	1.4134	1.4133	1.4124	1.4114	1.4090	1.4091
48.00	1.4192	1.4181	1.4154	1.4154	1.4145	1.4135	1.4110	1.4111
49.00	1.4213	1.4202	1.4174	1.4174	1.4167	1.4156	1.4131	1.4131
50.00	1.4235	1.4224	1.4195	1.4195	1.4189	1.4177	1.4151	1.4151
51.00	1.4257	1.4245	1.4215	1.4215	1.4210	1.4199	1.4171	1.4171
52.00	1.4279	1.4267	1.4236	1.4235	1.4232	1.4220	1.4192	1.4192
53.00	1.4301	1.4288	1.4256	1.4256	1.4254	1.4242	1.4212	1.4212
54.00	1.4324	1.4310	1.4278	1.4277	1.4276	1.4264	1.4233	1.4233
55.00	1.4346	1.4333	1.4299	1.4298	1.4298	1.4286	1.4254	1.4254
56.00	1.4369	1.4355	1.4320	1.4320	1.4321	1.4308	1.4275	1.4275
57.00	1.4392	1.4378	1.4341	1.4341	1.4344	1.4331	1.4296	1.4296
58.00	1.4415	1.4400	1.4363	1.4362	1.4367	1.4354	1.4318	1.4317
59.00	1.4438	1.4423	1.4384	1.4384	1.4390	1.4376	1.4339	1.4339
60.00	1.4462	1.4446	1.4406	1.4406	1.4413	1.4399	1.4361	1.4361
61.00	1.4486	1.4469	1.4428	1.4428	1.4437	1.4422	1.4383	1.4383
62.00	1.4510	1.4493	1.4450	1.4450	1.4461	1.4446	1.4405	1.4405
63.00	1.4534	1.4516	1.4473	1.4473	1.4485	1.4469	1.4427	1.4427
64.00	1.4558	1.4540	1.4495	1.4495	1.4509	1.4493	1.4449	1.4450
65.00	1.4582	1.4564	1.4518	1.4518	1.4534	1.4517	1.4471	1.4472
66.00	1.4607	1.4588	1.4540	1.4540	1.4558	1.4541	1.4494	1.4495
67.00	1.4631	1.4612	1.4563	1.4563	1.4583	1.4565	1.4517	1.4518
68.00	1.4656	1.4637	1.4587	1.4587	1.4608	1.4589	1.4540	1.4541
69.00	1.4681	1.4662	1.4610	1.4610	1.4633	1.4614	1.4563	1.4564
70.00	1.4707	1.4687	1.4633	1.4633	1.4658	1.4639	1.4587	1.4587
71.00	1.4733	1.4712	1.4657	1.4657	1.4683	1.4663	1.4610	1.4610
72.00	1.4759	1.4737	1.4681	1.4681	1.4709	1.4688	1.4634	1.4633
73.00	1.4785	1.4762	1.4705	1.4705	1.4735	1.4714	1.4657	1.4657
74.00	1.4811	1.4788	1.4729	1.4729	1.4761	1.4740	1.4681	1.4680
75.00	1.4837	1.4814	1.4754	1.4753	1.4787	1.4765	1.4706	1.4704
76.00	1.4863	1.4839	1.4778	1.4778	1.4813	1.4791	1.4730	1.4728
77.00	1.4890	1.4865	1.4802	1.4802	1.4840	1.4816	1.4754	1.4753
78.00	1.4917	1.4892	1.4827	1.4827	1.4867	1.4843	1.4778	1.4778
79.00	1.4944	1.4918	1.4852	1.4852	1.4895	1.4869	1.4803	1.4802
80.00	1.4971	1.4944	1.4876	1.4877	1.4922	1.4896	1.4828	1.4827
81.00	1.4998	1.4971	1.4901	1.4902	1.4949	1.4922	1.4853	1.4852
82.00	1.5026	1.4998	1.4927	1.4927	1.4976	1.4949	1.4878	1.4878
83.00	1.5054	1.5025	1.4953	1.4953	1.5004	1.4975	1.4903	1.4904
84.00	1.5082	1.5053	1.4978	1.4979	1.5031	1.5002	1.4929	1.4929
85.00	1.5110	1.5080	1.5004	1.5005	1.5059	1.5030	1.4955	1.4955
86.00	1.5138	1.5108	1.5030	1.5031	1.5087	1.5058	1.4981	1.4981
87.00	1.5166	1.5135	1.5056	1.5057	1.5115	1.5085	1.5008	1.5007
88.00	1.5195	1.5163	1.5083	1.5083	1.5143	1.5113	1.5034	1.5033
89.00	1.5224	1.5191	1.5109	1.5110	1.5172	1.5140	1.5060	1.5059
90.00	1.5252	1.5219	1.5136	1.5136	1.5202	1.5167	1.5087	1.5086

Experience has been that dilute sirups up to 35° B<sub>é</sub>. may be applied best by means of a dropping pipet controlled by a rubber bulb and that for Baumés above 35°, the sirup can be applied most conveniently by glass rods with fire-polished ends.

The procedure followed in obtaining a series of refractive indices over a temperature range was as follows:

After a satisfactory transfer of the sample, the readings were commonly taken by lowering the bath temperature to the lowest point and working upward. The bath was equipped with electrical heating coils and a steam inlet. Readings were made only after the precision thermometer on the refractometer had shown a constant reading for at least 5 minutes. In many instances, observations were continued for long periods to establish whether or not the earlier reading coincided with the latter, thus establishing equilibrium in the sirup or eliminating the possibility of mutarotation. Each sample was subjected to a series of transfers until successive readings agreed exactly in the fourth place. The transfers were made at room temperature or slightly higher, and the refractometer was adjusted to the desired temperature by the circulating water from the bath. It was found particularly advisable to transfer the lighter samples at room temperature or slightly higher, for if the circulating water is much below room temperature, the open prisms tend to fog by condensation of atmospheric moisture.

If it is desired to obtain refractive indices on heavy Baumé corn sirup at temperatures below 45° C., special precautions must be followed in handling the refractometer. The sirup should be applied warm and then cooled to the desired temperature. After the refractive index has been obtained, the prisms must be warmed before attempting their release. The seal of the prisms with heavy corn sirup at low temperatures is so great that they may be seriously damaged if a cold separation is attempted.

#### METHOD OF PLOTTING GRAPHS

The average number of refractive index-dry substance determinations for the four sirups—42.00, 55.00, 89.00, and 90.7 D.E.—was nineteen. Preliminary plotting had shown that the temperature differential in the range 18° to 50° C. could be considered a linear function for concentrations above 14% dry substance. For concentrations below 14% dry substance, the temperature relationship deviated from a linear function and approached that of water, with which it coincided at zero dry substance. Consequently, these four sirups were used for the master graphs at two temperatures: 20° and 45° C. The method of plotting was that of D.E. vs. factors (5). The factors for each experimental dry substance were obtained as follows:

$$\text{For } 20^{\circ} \text{ C.: } \frac{\text{Observed refractive index} - 1.3330}{\text{dry substance}}$$

$$\text{For } 45^{\circ} \text{ C.: } \frac{\text{Observed refractive index} - 1.3298}{\text{dry substance}}$$

The factors obtained ranged between 0.01300 and 0.02000. The graph obtained used dry substance as the ordinate, each millimeter equaling 0.10% D.S., and factors as the abscissas, each millimeter equaling 0.00002 factor value. The points were located on the graph paper by means of a hand lens and marked with the fine point of a drawing compass. The resulting curve drawn through the points in each case was of small, uniform curvature. From the four master curves, factor values were obtained for each dry substance and the refractive index was computed for these data.

The calculated values, when compared to the experimental data, gave the following results for each curve: Approximately 45% of comparative values were identical; approximately 40% differed by 0.0001



refractive index, this difference being equally divided between plus and minus values; approximately 10% differed by 0.0002 refractive index, again equally divided between plus and minus values; the remainder differed by 0.0003 to 0.0004 and were confined to the high dry substances range of 84 to 88%. Sirups of this dry substance are very viscous and the experimental technique is very difficult for the refractometer, Baumé, and moisture determinations. Several independent tests were always made at this range of dry substance in an attempt to "tie down" the refractive index-dry substance relationship. With ordinary plotting—i.e., refractive index *vs.* dry substance—it was difficult to distinguish valid data and the trend. The method of factors *vs.* dry substance clearly distinguished the valid data and defined the trend of the curve.

The resulting refractive index-dry substance data for the dry substance range of 0 to 90% and at temperatures of 20° and 45° C. appear in Table I.

Table II. Increase in Refractive Index Caused by 0.61% Added Ash

Dry Substance	Refractive Index at 45° C.
0.00	0.0000
10.00	0.00005
20.00	0.00010
30.00	0.00015
40.00	0.00020
50.00	0.00025
60.00	0.00030
70.00	0.00035
80.00	0.00040
90.00	0.00045

REFRACTIVE INDEX-DRY SUBSTANCE-DEXTROSE EQUIVALENT RELATIONSHIP

As stated earlier, the ash contents of the three sirups 42.00, 55.00, and 89.00 D.E. were essentially proportional ( $\frac{\text{ash content}}{\text{D.E.}} = K$ ) to the dextrose equivalent and any variation of the ash from this norm was less than the experimental error in measurement on a four-place refractometer. To obtain the effect of the dextrose equivalent on the refractive index, the values for the refractive index-dry substance for these three sirups were plotted in the following manner:

Refractive index was used for the ordinates, each millimeter equaling 0.0002, and dextrose equivalent for the abscissas, each millimeter equaling 0.20. Thus a cross plot of the three values would give the refractive index-dry substance relationship for a given dry substance value. The curve through the three points was found to be a straight line which did not deviate more than 0.0001 in refractive index values within the experimental limitations of the refractometer and the mechanics of plotting. The line for each dextrose equivalent was extrapolated to 30.00 and 92.00 D.E. From these data, the refractive indices for a 32.8 D.E. sirup were calculated for high dry substance values and compared to the experimental refractive index for the same sirup. The results were the same in most cases, with a deviation not greater than 0.0001 refractive index at any chosen value, again within the limitations of the refractometer and the graph paper. The same was true for the interpolated refractive index for 83.4 D.E. as compared to the experimental values. The facts indicate that the refractive index-dry substance-dextrose equivalent relationship can be obtained for any desired dextrose equivalent from these curves.

EFFECT OF ASH

The ash in starch conversion products is largely sodium chloride (5). In Table I essentially identical values for refractive index were obtained for the sugar sirups 89.00 D.E., ash 0.61% and 90.7 D.E., ash 1.22%.

This was pure coincidence, for the refractive index decrease with increasing dextrose equivalent and increases with increasing ash. However, if both sugar sirups are reduced to an ash-free dextrose equivalent basis, and recalculation made for the 89.0 D.E., 0.61% ash, to place it on the same dextrose equivalent basis as the 90.7 D.E., 1.22% ash, this calculated sugar would have the values 91.2 D.E., ash 0.61%. The refractive index of such sugar values can be obtained easily for all dry substance values shown above. The refractive index-dry substance values for this sugar in comparison to the values for the sugar sirup 90.8 D.E., ash 1.22%, immediately measures the effect of 0.61% ash for all dry substance values between 0 and 90%. A table for a sugar sirup of 91.2 D.E., ash 0.61%, has been constructed and the effect of ash content of 0.61% on the refractive index is given in Table II.

Since these data record the effect of one ash value for a complete range of dry substance, it is at once apparent that a complete set of ash corrections can be prepared with reasonable precision if at least one cross plot of ash effect can be obtained for high dry substance value. This is based on the assumption that the effect of ash is independent of the dextrose equivalent of the sirup.

A regular corn sirup was chosen to determine the effect of a (sodium chloride) because of ease of moisture determination and these former data may be consulted for the exact procedure. Briefly, a corn sirup substantially ash-free was prepared and salt was added to the sirup in increasing amounts. Two concentrations were so chosen that values above and below the desired constant dry substance were obtained with the corresponding refractive indices. A straight line cross plot for a fixed dry substance value was made in terms of refractive index. The values for a 76.34 D.S. sirup with increasing salt additions are given in Table III.

REFRACTIVE INDEX-COMMERCIAL BAUMÉ

Corn sirup is sold on a Baumé basis. This test requires special skill if reliable data are to be obtained. Therefore, a table giving the refractive index-commercial Baumé relationship is even more important from the standpoint of commerce than the

Table III. Increase in Refractive Index with Increasing Ash (76.34 D.S. sirup, refractive index at 45° C.)

Ash-Dry Substance Basis	Increase in Refractive Index
0.00	0.00000
1.00	0.00042
2.00	0.00086
3.00	0.00130
4.00	0.00174
5.00	0.00218
6.00	0.00250
7.00	0.00268
8.00	0.00274

Table IV. Commercial Baumé-Refractive Index

(Refractive indices of corn sirups and corn sugar sirups of various commercial Baumé values)											
Dextrose Equivalent-Ash											
Bé.	30.00	35.00	42.00	45.00	50.00	55.00	60.00	65.00	82.00	89.00	90.70
	0.28	0.28	0.28	0.28	0.30	0.30	0.30	0.30	0.41	0.41	0.41
Refractive Index at 45.00° C.											
40.00	1.4774	1.4773	1.4771	1.4770	1.4769	1.4768	1.4767	1.4766	1.4762	1.4762	1.4762
41.00	1.4825	1.4824	1.4822	1.4821	1.4820	1.4820	1.4818	1.4817	1.4813	1.4813	1.4813
42.00	1.4878	1.4877	1.4875	1.4874	1.4873	1.4873	1.4871	1.4869	1.4865	1.4865	1.4865
43.00	1.4933	1.4931	1.4929	1.4928	1.4927	1.4926	1.4924	1.4923	1.4919	1.4919	1.4919
44.00	1.4986	1.4985	1.4983	1.4982	1.4981	1.4980	1.4978	1.4977	1.4973	1.4973	1.4973
45.00	1.5041	1.5040	1.5038	1.5037	1.5036	1.5036	1.5034	1.5033	1.5029	1.5029	1.5029
46.00	1.5098	1.5097	1.5095	1.5094	1.5093	1.5092	1.5090	1.5089	1.5085	1.5085	1.5085
47.00	1.5155	1.5154	1.5152	1.5151	1.5150	1.5149	1.5148	1.5147	1.5143	1.5143	1.5143
Refractive Index at 20.00° C.											
40.00	1.4825	1.4824	1.4821	1.4820	1.4819	1.4818	1.4817	1.4815	1.4810	1.4810	1.4810
41.00	1.4876	1.4875	1.4872	1.4871	1.4870	1.4869	1.4868	1.4866	1.4862	1.4862	1.4862
42.00	1.4928	1.4927	1.4925	1.4924	1.4923	1.4921	1.4920	1.4918	1.4913	1.4913	1.4913
43.00	1.4981	1.4980	1.4978	1.4977	1.4976	1.4975	1.4973	1.4971	1.4967	1.4967	1.4967
44.00	1.5037	1.5035	1.5033	1.5032	1.5031	1.5030	1.5029	1.5027	1.5022	1.5022	1.5022
45.00	1.5093	1.5092	1.5089	1.5088	1.5087	1.5086	1.5084	1.5082	1.5077	1.5077	1.5077
46.00	1.5148	1.5147	1.5145	1.5144	1.5143	1.5142	1.5141	1.5139	1.5135	1.5135	1.5135
47.00	1.5208	1.5206	1.5204	1.5203	1.5202	1.5201	1.5200	1.5198	1.5193	1.5193	1.5193



refractive index-dry substance table. Such a table was constructed by the following procedure:

The refractive index was obtained at two temperatures, 20.00° and 45.00° C., for the dry substance corresponding to the commercial Baumés 40 to 47 according to the accepted values for this relationship (5). The three master graphs, 42.00, 55.00, and 89.00 D.E., were employed. These data were plotted on millimeter paper employing refractive index as the ordinate, each millimeter equaling 0.0002 refractive index, and as abscissa, dextrose equivalent, each millimeter equaling 0.10 D.E. Thus each set of points represented one Baumé and a straight line was found to be the best curve through these points. The deviation of points from the straight line was not greater than 0.0001 refractive index—i.e., within the limits of the refractometer and the graph paper. The curve was extrapolated to 30.00 and 100.00 D.E. The extrapolated values for 32.8 D.E. were either equal to or not greater than 0.0001 refractive index from the experimental values for the sirup, as were also the interpolated values for the 83.4 D.E. sugar sirup. From the graph of these values, tables of refractive index-commercial Baumé for a range of dextrose equivalents have been calculated (Table IV). A column of values is also given for the corn sugar sirup—90.7 D.E., 1.22% ash—which, because of its ash content, is not covered by the above method of graphing but is of interest because such a corn sugar sirup is typical of that required for an "80" sugar.

#### SUMMARY

The refractive index of starch conversion products decreases with increasing dextrose equivalent. The effect of ash (sodium chloride) is to increase the refractive index. The decrease of

refractive index with increasing dextrose equivalent is proportional to the increase in dextrose equivalent if the ash content of the products is also proportional to the dextrose equivalent.

The temperature-refractive index relationship is linear within the range 18° to 45° C. when the concentration exceeds 14% dry substance and within the limits of precision of a four-place instrument.

Tables are presented covering the refractive index-dry substance relationship for starch conversion products typical of commerce, and refractive index-commercial Baumé for typical commercial products of varying dextrose equivalents.

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## Determination of Zinc in Cyanide Brass-Plating Baths

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THE large number of papers on the determination of zinc which have appeared during the past 8 years attest to the widespread need for rapid and reliable analytical procedures for the determination. Of the many methods available for the determination of zinc, only two appear to meet the demands placed on control methods. A control method does not long survive unless it is inherently reliable, rapid, and inexpensive. The methods found to possess these qualities are: (1) the ferrocyanide titration using diphenylbenzidine as internal indicator with ferric ion held in solution as the pyrophosphate complex and (2) electrodeposition (5). Where electrolytic equipment is largely tied up by copper determinations, the determination of zinc by ferrocyanide titration is designated. The application of this method to cyanide brass-plating solutions is discussed below. The application of the electrolytic determination of zinc will be discussed in a later paper. Recently (4) the polarograph has been successfully employed for determination of the copper-zinc ratio of electrodeposited brass. The authors do not as yet report its use in the determination of copper and zinc in the brass-plating bath.

#### PROCEDURE

Centrifuge until clear a portion of the cyanide brass-plating solution to be analyzed, pipet 10 ml. into a 180-ml. electrolytic beaker, add dropwise 10 ml. of concentrated hydrochloric acid, heat until clear. If the solution is not heated until clear, ferrocyanides initially precipitated by the acid will not completely redissolve in the next step. Cool and carefully add 7 ml. of a mixture consisting of 3 volumes of concentrated nitric acid and 2 volumes of concentrated sulfuric acid. Evaporate to sulfur dioxide fumes, finally heating over an open flame to cause copious fuming. This step appears essential to assure complete destruction of ferrocyanides and of organic matter. Cool the residual salts, wash the beaker sides, add 1 ml. of 1 to 1 nitric acid, remove copper by electrodeposition. After the removal of copper, add 3 drops of 5% ammonium

persulfate solution to oxidize any ferrous iron to the ferric state. Evaporate to 25 ml. to destroy excess persulfate. Cool, rinse beaker walls, add 2 grams of sodium pyrophosphate to form the ferric iron complex, and finally, 5 ml. of concentrated ammonia. At this point the solution should be alkaline. If not, make just alkaline to phenolphthalein. Neutralize the solution with 1 to 1 sulfuric acid and then add 6 ml. in excess. Warm the solution to 40° to 45° C. At this stage commence vigorous mechanical agitation of the solution. This is essential for the rapid response of the indicator to ferrocyanide additions. Add 3 drops of 1% diphenylbenzidine in sirupy phosphoric acid and 3 drops of 0.2% potassium ferricyanide. The latter addition causes the formation of the violet oxidation product of diphenylbenzidine. Titrate to a permanent end point, using a 0.025 molar solution of potassium ferrocyanide containing 0.3 gram of potassium ferricyanide per liter. The ferricyanide in the titrating solution is essential for obtaining reproducible results and for maintaining good indicator reactivity.

#### LIMITATIONS AND ERRORS

The successful functioning of the internal indicator requires careful control of the solution composition. However, the determination is not subject to all the limitations cited in the literature. Nitrates can be present to the extent of 0.5 ml. of concentrated nitric acid per 50 ml. of solution. This makes readily possible the use of small amounts of nitric acid as cathode depolarizer in the electrodeposition of copper. The indicator functions properly in a solution containing 4.5 grams of ammonium sulfate, 6 ml. of 1 to 1 sulfuric acid, and at least 20 mg. of zinc ion per 50 ml. of solution. These proportions must be maintained in order to preserve the sensitivity of the indicator. Samples containing less than 20 mg. of zinc ion may require the addition of known amounts of standard zinc chloride solution prepared from metallic zinc.

To determine the effect of extraneous substances in concentrations higher than those encountered in the analysis of a typical plating solution, a standard zinc chloride solution was used.



Table I. Effect of Extraneous Substances on Diphenylbenzidine End Point

Substance (Plus Blank)	Ml. of 0.025 M Potassium Ferrocyanide	Effect on Indicator Color Change
Blank (21.6 mg. of Zn <sup>++</sup> in approximately 50 ml. of soln.)	8.85	Yellow-green end point color change
10 mg. of Pb <sup>++</sup>	8.85	No effect
10 mg. of As <sup>+++</sup>	8.85	No effect
10 mg. of Sb <sup>+++</sup>	8.85	No effect
10 mg. of Sn <sup>++</sup>	8.85	No effect
10 mg. of Al <sup>+++</sup>	8.85	Slightly slower color change
20 mg. of Fe <sup>+++</sup>	8.85	Blue-green end point
(as pyrophosphate complex)		
40 mg. of Fe <sup>+++</sup>	8.85	Blue-green end point
(as pyrophosphate complex)		
60 mg. of Fe <sup>+++</sup>	8.85	Blue-green end point
(as pyrophosphate complex)		
10 mg. of Ni <sup>++</sup>	15.05	(Ni precipitated as the ferrocyanide)
10 mg. of Th <sup>+</sup>	8.85	No effect
10 mg. of Mg <sup>++</sup>	8.85	No effect
10 mg. of Ca <sup>++</sup>	8.85	No effect
10 mg. of SiO <sub>3</sub> <sup>--</sup>	8.85	No effect
20 mg. of SiO <sub>3</sub> <sup>--</sup>	8.85	Color change slow
4 ml. of 1 to 1 HNO <sub>3</sub>	9.15	No change
2.5 ml. of 1 to 1 HNO <sub>3</sub>	8.90	No change
1.3 ml. of 1 to 1 HNO <sub>3</sub>	8.85	No change
10 mg. of Durodex cleaner <sup>a</sup>	8.85	Color change less sharp
10 mg. of Magnus cleaner <sup>b</sup>	8.80	Color change less sharp
10 mg. of gelatin	8.85	No effect
10 mg. of thioglycol	...	No color or end point
10 mg. of acid pickling inhibitor <sup>c</sup>		
1	...	No color or end point
2	...	No color or end point
3	...	No color or end point

<sup>a</sup> An alkaline cleaner with about 10% silicate and 0.5% phosphate.

<sup>b</sup> Similar to Durodex but containing a small amount of soap.

<sup>c</sup> The three types of inhibitors used are thought to be:

1, a piperidine derivative.

2, a sulfonated primary or secondary aromatic amine.

3, an aldehyde-aromatic amine reaction product.

Each titration sample was prepared by pipetting 10 ml. of standard zinc chloride solution into a 180-ml. electrolytic beaker, adding 4 grams of ammonium sulfate, 6 ml. of 1 to 1 sulfuric acid, and finally the extraneous material to be tested. The resulting solution was diluted to 50 ml., heated to 45° C., and titrated with the ferrocyanide solution.

A common source of indicator trouble arises from the presence of small amounts of surface-active organic matter in the solution. Reference to Table I shows that such material is capable of preventing completely the formation of the colored form of the indicator and must as a consequence be destroyed before proceeding to the zinc titration. Certain common inorganic ions in concentrations as high as 0.2 mg. per ml. of solution do not interfere significantly with the titration. The end point is unaffected and

the color change remains sharp, though slight changes in hue may result. A silicate-ion concentration above 0.5 mg. per ml. renders the color change slow and less distinct. In the absence of iron the end point color is yellow-green; in the presence of iron, blue-green. A few trials with solutions of known zinc content will familiarize the analyst with the various stages of the color changes before and at the end point. However, a few hints will aid (3).

With the initial addition of 3 drops of ferricyanide, a violet color should develop. Lack of color at this point indicates serious divergence from the suggested procedure. A new sample is indicated. With the addition of 0.025 molar potassium ferrocyanide (containing 0.3 gram of potassium ferricyanide per liter), the initial violet changes to blue. As the titration proceeds, the blue color fades to a light shade of blue. At a few milliliters from the end point, this light blue will change through blue-green, then yellow-green, and finally attains a light violet color. The color transitions obtained following the light blue stage depend on the rate at which ferrocyanide is added, on the temperature, and on the rate of stirring. Some stages in the color change may not appear. The color at about 2 ml. from the end point should, however, be violet. Enough time must be allowed for the development of the violet complex, which at 40° to 45° C. is a very sensitive and mobile indicator.

If sufficient time is allowed for its development after each increment of ferrocyanide, the end point can be approached with certainty and precision. The titration requires 5 to 10 minutes. Starting with clear bath solution, the determination of both copper and zinc requires an average of 1.5 hours. A distinct advantage of the suggested procedure is that no transfers are required, the analysis being started and finished in the same vessel.

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## Determination of Sesamin

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A method for the quantitative determination of sesamin in sesame oil is based upon measurement of the greenish-yellow color produced by sesamin when it is allowed to react with a mixture of perchloric acid and hydrogen peroxide.

**S**ESAME oil, a vegetable oil extensively used in Europe and Asia for culinary purposes, has commanded but little attention in this country, and the number of published American investigations (7) on it is small. In a large measure this is probably because it is an imported oil and is not an important article of our commerce.

In 1940 Eagleson (1, 3) showed that the toxicity to houseflies of a kerosene solution of pyrethrins was considerably increased by the addition of a small amount of sesame oil. The oil

alone in kerosene was without effect and was the only one of animal and vegetable oils tested (2) that produced synergism. By fractional distillations of sesame oil Haller *et al.* (5) showed that the principle responsible for this synergistic effect is sesamin, one of the components of the nonsaponifiable fraction and characteristic constituent of the oil. Sesamin is a substituted bicyclodihydrofuran and is not very reactive chemically. It can also be removed from the oil by extraction with 90% acetic acid, or by adsorption (6) on charcoal or clay, from which it can be removed by elution with suitable solvents.

Besides sesamin, sesame oil contains sesamol, which on treatment with mineral acid yields sesamol, a phenol. This compound is responsible for several of the color tests (8, 9) used to identify sesame oil. Its value as a synergist with pyrethrins is not known.



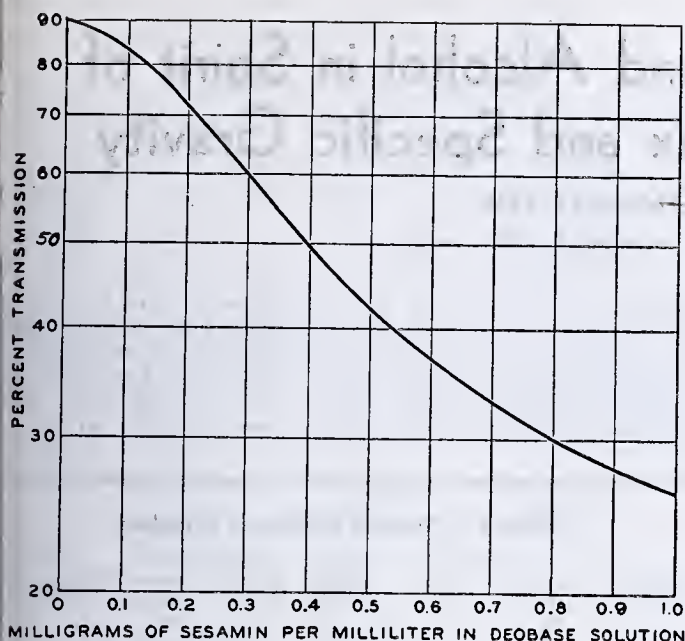


Figure 1. Standard Reference Graph

With the curtailment of pyrethrum imports due to the war, the discovery that sesame oil containing sesamin could save part of the pyrethrins in insecticidal compositions has assumed considerable importance. The addition of 5% of sesame oil to a solution of pyrethrins in kerosene (2) or dichlorodifluoromethane (4) saves about 50% of pyrethrum.

To be effective as a synergist with pyrethrum, sesame oil must contain sesamin, but the minimum quantity needed has not yet been determined because the sesamin content is variable, and no quantitative method has been proposed for its detection and estimation. Whether the variability is due to variation in the seed or to removal of more or less of the sesamin in the process of refining the oil also remains to be determined.

This paper proposes a method for the determination of sesamin, based upon the observation that a mixture of perchloric acid and hydrogen peroxide gives a greenish-yellow color with vegetable oil containing sesamin. The color persists for about 6 minutes and this allows ample time for measurement. A curve is established, from which the sesamin content of unknown solutions is read directly, by measuring the color produced by standard solutions of pure sesamin dissolved in sesame-free sesame oil. The color is measured in this laboratory in an Aminco photoelectric photometer employing a No. 46 blue filter (optical centroid at about 460 millimicrons) and water as a blank to balance the photometer at 100% transmission. (Formation of bubbles of oxygen, after 10 to 15 minutes, in reagent-refined kerosene mixture precludes its repeated use as the blank.) In carrying out the following procedure for preparing the standards and the reagent and for developing the color, it is believed that reasonably accurate results may be obtained.

#### PROCEDURE

**PREPARATION OF REAGENT.** Caution must be exercised in handling the following reagents, since they are extremely corrosive. Add, with shaking, 2 ml. of 30% hydrogen peroxide to 10 ml. of 70 to 72% perchloric acid maintained at 15° to 20° C. A mixture that is prepared below 10° C. gives erratic transmission values. This amount of reagent suffices for one reading only. The reagent must be made up fresh for each reading not more than 10 minutes before use. If allowed to stand longer, it solidifies rapidly and also gives erratic values.

**PREPARATION OF STANDARD SOLUTIONS.** The sesamin-free sesame oil used in the standard solutions is prepared by dissolving 100 grams of sesame oil in 100 ml. of petroleum ether (b. 30° to 60° C.) and extracting it in a separatory funnel about six times with 20-ml. portions of 90% acetic acid, or until most portion of the oil shows no color in the reagent described above. The petroleum ether solution of the oil is washed free of acid with water and then dried over sodium sulfate. The oil

is recovered in 97% yield by removing the solvent completely under reduced pressure.

The pure sesamin used in the standard solutions may be obtained by washing the combined acetic acid extracts once with 25 ml. of petroleum ether and then removing almost all the acetic acid under reduced pressure, at 15 to 20 mm., 60° to 80° C. The residue crystallizes when 5 to 10 ml. of hot 95% ethanol are added. The sesamin is filtered from the cold solution and purified by several recrystallizations from ethanol. The sesamin used in these determinations melted at 121–122° C. (corrected). All samples of refined sesame oil tested in this laboratory were found by acetic acid extraction to contain approximately 1% of sesamin by weight.

One hundred milligrams of pure sesamin, ground in a mortar to a fine powder, are made up to 10 grams with the sesamin-free sesame oil and dissolved with slight warming. Aliquots of 1000, 750, 500, 250, and 100 mg. of this solution are made up to 10 ml. with refined kerosene in separate volumetric flasks. (In these experiments the kerosene used was Deobase.) These standard sesame oil solutions therefore contain 1.00, 0.75, 0.50, 0.25, and 0.10 mg. of sesamin per ml., respectively.

**DEVELOPMENT AND MEASUREMENT OF COLOR.** One milliliter of the standard solution is pipetted into a small, dry centrifuge tube. The 6 ml. of freshly prepared reagent are added, and the tube is closed quickly with a clean, tight-fitting rubber stopper. After being shaken vigorously for 30 seconds, the tube is centrifuged for 2 minutes to clear the resulting emulsion, and then the contents are carefully poured into a clean, dry photometer test tube. The aqueous layer, at the bottom of the tube, shows a color ranging from faint yellow to dark greenish yellow, depending upon the amount of sesamin present. The color is measured in the photometer exactly 5 minutes after the reagent is added. The procedure is repeated on each of the other standard solutions. As shown in Figure 1, the results are plotted on semilogarithmic paper as per cent transmission against concentration of sesamin, providing a standard graph for reference of all analyses made on the same instrument with the same reagents.

To determine the sesamin content of a sample of unknown oil, usually 500 mg. of oil are diluted to 10 ml. with refined kerosene. The 500-mg. sample is ideal for any oil containing from 0.25 to 1.75% of sesamin.

It is probably best to keep the transmission readings between 80 and 28% by adjustment, if necessary, of the size of sample taken. A 1-ml. aliquot of this solution is pipetted into a dry centrifuge tube and the procedure outlined above for developing the color in the standard solutions is followed exactly. The percentage of light transmission is measured in the photometer and is referred to the standard graph. The concentration of sesamin contained in the aliquot of the kerosene solution is read from the standard graph, and the percentage of sesamin in the unknown oil is calculated as follows:

$$\text{Per cent of sesamin} = \frac{\text{concentration of sesamin} \times 10}{\text{milligrams of unknown oil}} \times 100$$

#### PRECISION OF METHOD

The curve shown in Figure 1 is drawn through 6 points, each of which is the average of 10 measurements. The standard deviations of the transmission readings tended to increase with the readings themselves, the largest deviation,  $\pm 2.5\%$ , occurring at 65% transmission. Allowance for this trend and for the curvature of the standard line indicates that the standard error of the final result is practically constant, and that it amounts to about  $\pm 0.05\%$  of sesamin when the result of a single determination on an unknown is read from a curve established from 10 replicates.

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# Determination of Camphor and Alcohol in Spirit of Camphor by Refractive Index and Specific Gravity

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IN EARLIER investigations the authors accomplished the analysis of spirit of camphor by means of 2,4-dinitrophenylhydrazine (1) and the optical activity of camphor (2). The method presented here uses the two physical constants, refractive index and specific gravity, and is applicable to synthetic camphor as well as the natural product.

Schoorl (3) and Weber (4) used physical constants for the analysis of spirit of camphor official in the Dutch and German Pharmacopoeias, respectively. However, their works are not applicable to analysis of the United States Pharmacopoeial product because the preparations in the Dutch and German Pharmacopoeias contain 10% (by weight) camphor and about 60% (by weight) alcohol. The composition of those spirits therefore varies considerably from that of the U.S.P. product.

## EXPERIMENTAL

Samples of camphor were obtained from widely different sources over a period of 10 years. Each sample was purified at least twice by sublimation. The first 10% and the last 5% of the sample were rejected each time.

Several solutions containing varying percentages of camphor and of alcohol were prepared from each sample. The solutions studied were made to contain from 7.5 to 11.5% (weight to volume) camphor and from 70 to 90% (by volume) alcohol by the method described in a previous communication (2).

The refractive indices of the solutions were determined at 20° C. with a Valentine, Abbe-type refractometer and the specific gravities were determined with a 25-cc. pycnometer at 20°/20° C.

From the refractive indices and the specific gravities of about 100 solutions a chart (Figure 1) was constructed in which the ordinates represent the refractive indices and the abscissas represent the specific gravities. By connecting the points as

plotted, several four-sided figures were formed and divided in smaller units in order to facilitate the use of the chart. Greater accuracy can be obtained with an enlarged chart with additional subdivisions. The more nearly horizontal lines represent camphor percentages from 7.5 to 11.5 and the more nearly vertical lines represent alcohol percentages from 70 to 90.

Table I. Analysis of Natural Camphor

Camphor Present %	Alcohol Present %	Refractive Index	Specific Gravity	Camphor Determined %	Variation %	Alcohol Determined %
7.50	70.00	1.3719	0.8780	7.50	0.00	70.00
7.50	87.93	1.3715	0.8237	7.41	-0.09	87.80
7.50	90.00	1.3708	0.8153	7.49	-0.01	89.97
7.75	80.00	1.3726	0.8503	7.60	-0.15	79.75
7.85	77.00	1.3727	0.8580	7.75	-0.10	77.08
7.92	73.00	1.3726	0.8695	7.90	-0.02	73.08
8.00	86.00	1.3724	0.8297	7.99	-0.01	85.94
8.75	78.00	1.3738	0.8538	8.74	-0.01	78.05
8.80	74.00	1.3736	0.8658	8.72	-0.08	73.98
8.90	80.00	1.3739	0.8479	8.87	-0.03	80.00
9.00	88.00	1.3726	0.8194	9.05	+0.05	88.00
9.35	82.50	1.3742	0.8388	9.38	+0.03	82.47
9.40	83.00	1.3743	0.8365	9.58	+0.18	83.19
9.75	87.50	1.3732	0.8199	9.64	-0.11	87.52
9.80	85.00	1.3743	0.8300	9.92	+0.12	84.87
10.00	70.00	1.3747	0.8751	10.05	+0.05	69.97
10.00	74.00	1.3750	0.8640	9.94	-0.06	74.00
10.00	80.00	1.3751	0.8467	10.00	0.00	79.90
10.00	80.00	1.3751	0.8465	10.00	0.00	79.89
10.00	80.50	1.3750	0.8440	9.97	-0.03	80.64
10.00	85.31	1.3742	0.8285	9.93	-0.07	85.28
10.00	85.31	1.3743	0.8280	10.06	+0.06	85.30
10.00	85.31	1.3743	0.8287	10.03	+0.03	85.20
10.00	85.31	1.3744	0.8286	10.11	+0.11	85.12
10.00	86.00	1.3740	0.8250	10.02	+0.02	86.06
10.20	83.00	1.3749	0.8364	10.13	-0.07	82.84
10.60	76.00	1.3757	0.8572	10.46	-0.14	76.20
10.90	82.00	1.3756	0.8375	10.77	-0.13	82.12
11.00	75.00	1.3764	0.8601	11.10	+0.10	74.85
11.00	84.00	1.3754	0.8300	10.96	-0.04	84.20
11.10	87.00	1.3746	0.8188	11.09	-0.01	86.86
11.50	70.00	1.3764	0.8730	11.58	+0.08	70.00
11.50	85.00	1.3758	0.8257	11.55	+0.05	85.11

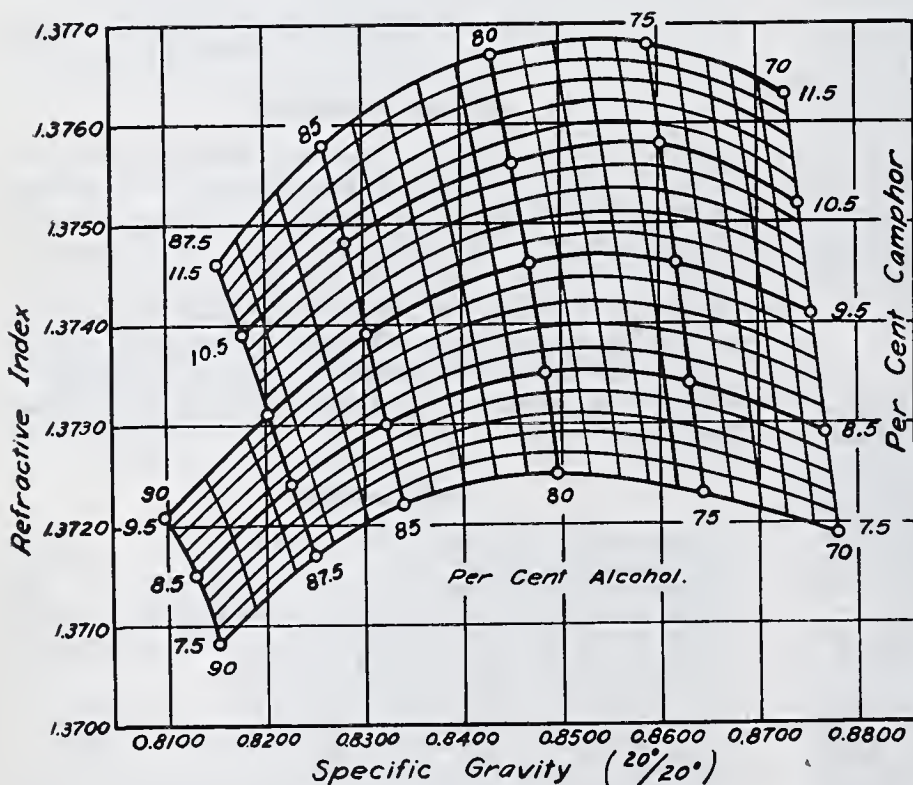


Figure 1. Determination of Camphor and Alcohol

The chart was used for the analysis of samples of spirit of camphor of known composition. The refractive index and specific gravity of each solution were determined. The point of intersection on the chart found from these constants was located by reference to the respective ordinate and abscissa. From this point of intersection the percentage of alcohol was determined by following along the nearly vertical lines from the point of intersection to the outside of the four-sided figure, either top or bottom. The percentage of camphor was determined following the nearly horizontal lines to the side, either right or left. For example, the refractive index of 1.3740 and the specific gravity of 0.8500 may be selected. After the point of intersection has been found, the alcohol content may be read as 79.29% and the camphor content as 8.92%.

Thirty-three samples of spirit of natural camphor of known composition were analyzed by use of a chart similar to Figure 1; the results are given in Table I. The determined values are in accord with the theoretical values. The camphor content shows variations of -0.15 to +0.18% and the alcohol content of -0.25 to +0.20%.

Since synthetic camphor is now used to a considerable extent, several samples of synthetic spirit of camphor were prepared with the synthetic product



Table II. Analysis of Synthetic Camphor							
Camphor Present %	Alcohol Present %	Refractive Index	Specific Gravity	Camphor Determined %	Variation %	Alcohol Determined %	Variation %
10.00	85.56	1.3741	0.8275	9.92	-0.08	85.50	-0.06
10.00	85.56	1.3742	0.8279	9.98	-0.02	85.35	-0.21
10.00	85.56	1.3741	0.8277	9.90	-0.10	85.48	-0.08
10.15	77.00	1.3754	0.8549	10.15	0.00	77.00	0.00
10.00	85.56	1.3743	0.8278	10.08	+0.08	85.34	-0.22
10.40	84.00	1.3749	0.8321	10.33	-0.07	83.88	-0.12
9.70	79.00	1.3749	0.8501	9.75	+0.05	78.91	-0.09
10.00	85.56	1.3742	0.8278	9.98	-0.02	85.43	-0.13
10.00	86.00	1.3741	0.8250	10.03	+0.03	85.94	-0.06
9.30	84.00	1.3739	0.8341	9.31	+0.01	83.98	-0.02
10.00	85.54	1.3744	0.8277	10.17	+0.17	85.33	-0.21
11.10	81.00	1.3763	0.8405	11.22	+0.12	80.93	-0.07
8.00	80.00	1.3730	0.8486	8.00	0.00	80.15	+0.15
10.00	85.56	1.3742	0.8277	10.00	0.00	85.45	-0.11
10.90	80.00	1.3760	0.8447	10.85	-0.05	80.00	0.00
10.00	85.40	1.3743	0.8284	10.03	+0.03	85.18	-0.22
10.00	80.00	1.3751	0.8467	10.00	0.00	79.90	-0.10
10.50	85.60	1.3746	0.8250	10.54	+0.04	85.70	+0.10
10.00	85.40	1.3742	0.8286	9.91	-0.09	85.20	-0.20
10.00	86.00	1.3741	0.8250	10.07	+0.07	85.97	-0.03
9.70	79.00	1.3749	0.8501	9.75	+0.05	78.91	-0.09
10.00	85.40	1.3742	0.8286	9.91	-0.09	85.20	-0.20
10.50	85.60	1.3745	0.8250	10.45	-0.05	85.77	+0.17
9.70	79.00	1.3750	0.8500	9.82	+0.12	78.78	-0.22

lected from eight different sources; Table II shows the results of the analyses. Again these are in accord with the theoretical values.

SUMMARY

A chart has been constructed from which the percentages of alcohol and camphor in spirit of camphor may be determined when the refractive index and specific gravity of the spirit are known. Analyses of different samples of spirit of camphor, whether prepared from natural or synthetic camphor, by the proposed method show close agreement with the theoretical values.

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# Precision and Accuracy of Colorimetric Procedures as Analytical Control Methods

## Determination of Aluminum

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Colorimetric procedure for the determination of aluminum, calculated and represented as aluminum trioxide and involving the formation of the red complex by the interaction of the aluminon reagent with the aluminum ion, has been developed to meet the special requirements in the rapid analysis of leach liquors in pilot-plant operations. The factors influencing color intensities have been investigated and the requisite techniques for a precision and accuracy of a control character are described. Employing these techniques in the analysis of an aliquot of the leach liquor, precision and accuracy studies as applied to ordinary and refined laboratory techniques have been made on typical analytical data. Statistical reasoning based on the standard deviation is applied to the acquired data. Applying ordinary laboratory techniques, the average precision, measured by the average deviation of the single results from mean, is of the order of 1% or 10 parts per 1000, while the over-accuracy is of the order of 1 to 3%.

NUMEROUS literature references (7, 8, 12) and recently published books (3, 6, 17) describe in detail the procedures involved in colorimetric determinations. Although the colorimetric method has been used for the rapid estimation of small quantities of many inorganic substances, not a great deal of emphasis has been placed on the precision and accuracy that might be expected in its use as an analytical control method. In quality control work, speed is so essential that precision and accuracy are often sacrificed; however, since intelligent conclusions in pilot-plant operations have to be based on the analytical data, it is essential to ascertain the precision and accuracy of the control methods. Recent investigations in this laboratory have been concerned with the colorimetric procedures involving aluminum, titanium, iron, and sodium. The procedures are, for the most part, adaptations of previously published methods; however, as a

matter of convenience, deviations from standard procedures are necessarily made from time to time, and the subsequent effects of the variables on precision and accuracy are briefly considered. Statistical reasoning based on the standard deviation is applied to the acquired data (1). The purpose of this investigation is therefore twofold: to describe satisfactory laboratory techniques in colorimetric procedures as applied to aluminum and to evaluate the precision and accuracy that might be expected in routine analyses.

The usual procedure in the colorimetric determination of aluminum involves the formation of the red complex by the interaction of the ammonium salt of aurin tricarboxylic acid (aluminon) and the aluminum ion in a carefully buffered solution (3). In the investigation of aluminum in plants, Winter, Thrun, and Bird (15) conclude that maximum color is obtained in the presence of 10% ammonium acetate when the solution is maintained at a temperature of 80° C. for 10 minutes and pH 4 (approximately). In the presence of 25 ml. of both ammonium acetate and ammonium chloride, they find that the dye changes color at about pH 7. Roller (10) states that the red color which aluminum ion gives with aurin tricarboxylic acid is much more sensitive if made at about pH 6.3 instead of in alkaline solutions as recommended by Yoe and Hill (16). The latter authors, investigating the procedure under different experimental conditions, cite five factors that affect the test for aluminum with aluminon: time, temperature, volume, concentration, and the presence of other ions. Lampitt, Sylvester, and Belham (5) suggest the use of glycerol to stabilize the lake formed. Thrun (13) has investigated the use of protective colloids in colorimetric determination of certain metals as lakes of dyes and recommends the use of a gum arabic solution to keep the aluminum lake of aurin tricarboxylic acid in solution.

The colorimetric method presented here for the determination of aluminum, calculated and represented as aluminum trioxide, has been developed at this station to meet the special requirements in the rapid analysis of leach liquors in pilot-plant operations. The sample taken for analysis must be free from inter-



Table I. Colorimeter Readings

Test Tube	(0.04 mg. of $\text{Al}_2\text{O}_3$ )		Average
	No. 1	No. 2	
1	185	185	185
2	185	185	185
3	185	186	186
4	184	186	185
5 <sup>a</sup>	161	166	164
1a <sup>a</sup>	173	168	170
2a <sup>a</sup>	159	157	158
3a <sup>a</sup>	183	178	181
4a <sup>a</sup>	158	158	158
5a <sup>a</sup>	163	163	163
1b	187	186	187
2b	183	186	185
3b	184	187	186
4b	184	187	186
5b	185	187	186

<sup>a</sup> Old test tubes; previous history unknown.

fering ions, which include ferric iron, beryllium, and chromium, since these form a lake similar to that formed by aluminum. Certain variations may be introduced to eliminate these interferences. Chromium lake, for instance, in acetate solution is rapidly decomposed by the addition of ammonia and ammonium carbonate (6). Several procedures may be followed for eliminating interfering iron (4, 9). Phosphate, if present in appreciable quantities, prevents the formation of aluminum lakes.

#### ANALYTICAL PROCEDURE

**REAGENTS. Composite Solution.** Dissolve 154 grams of ammonium acetate, 5 ml. of concentrated hydrochloric acid, 0.400 gram of ammonium salt of aurin tricarboxylic acid and 1 gram of gum arabic in water, and dilute to 1000 ml. Dissolve each reagent in a minimum quantity of distilled water, and add the ingredients of the composite in the order named. The aluminum reagent, weighed out to the nearest milligram, dissolves readily in cold water. To make accurate dilutions, the solution of gum arabic must be cautiously added; otherwise persisting foams will greatly alter the liquid level. The composite solution deteriorates with age, especially when exposed to the light; it therefore, must be protected from light when stored.

**Standard Aluminum Solution.** Dissolve 4.74 grams of aluminum chloride hexahydrate in 1000 ml. of water (1 ml. = 1 mg. of aluminum trioxide) and standardize gravimetrically (2).

**Working Standard.** Dilute 5 ml. of the standard to 500 ml. (1 ml. = 0.01 mg. of aluminum trioxide).

**PROCEDURE.** Discharge an aliquot of the previously diluted and acidified leach liquor (10 ml. of liquor and approximately 15 ml. of concentrated hydrochloric acid in 250 ml.), of an amount estimated to contain 0.01 to 0.06 mg. of aluminum trioxide, into a 25-ml. calibrated blood-sugar test tube by means of a pipet, add distilled water to the 12.5-ml. mark, and thoroughly mix the contents. Add 10 ml. of the composite solution by means of an automatic pipet and sufficient water to bring the meniscus to the 25-ml. mark. Mix the contents of the tube well and place in a boiling water bath for precisely 10 minutes. Cool the tube and contents in running tap water for 5 minutes, mix again, and determine the color absorption with the Klett-Summerson photoelectric colorimeter. A filter of range 500 to 570 millimicrons is employed, since a spectrophotometric study of the color in question shows a maximum absorption at 530  $m\mu$  in the red complex.

Since absorption of the red color is not a linear function of the aluminum trioxide concentration, a calibration curve must be established. Quantities of the working standard, equivalent to 0.01, 0.02, 0.03, 0.04, 0.05, and 0.06 mg. of aluminum trioxide, are discharged into blood-sugar test tubes, and the lakes are formed in the usual manner.

Procedures for the gravimetric analysis of aluminum involve the use of a modification of the quinolate method (2).

From the standpoint of accuracy, ease of manipulation, and rapidity of technique, preliminary investigations indicated that the method of dilution was entirely satisfactory. Calibration of twenty blood-sugar test tubes resulted in an average precision of 0.2% or 2 parts per 1000. No detectable difference in colorimeter reading could be observed in comparing procedures involving pipets, burets, and 25-ml. blood-sugar test tubes.

In an investigation on the factors influencing color intensities, certain anomalous results were obtained in establishing the standardization curve (Table I). Even though these tubes were

thoroughly cleaned with chromic acid cleaning solution, it is apparent that only new tubes gave reproducible results. The previous history of the remainder of tubes was unknown. Table I shows the effects of chemically clean test tubes on the reproducibility factor. Tubes in Series I of this table were cleaned with chromic acid cleaning solution; tubes of Series II were cleaned by treating successively with chromic acid, water, ethyl alcohol, benzene, and water; and tubes of Series III were treated with hot chromic acid, water, and alkaline cleaning mixture (14) and rinsed thoroughly with distilled water. Thus, to obtain reproducibly accurate results the test tubes must be chemically clean. In all subsequent colorimetric measurements, new tubes only are used, and these are cleaned, using the procedure as established for Series III.

The length of time in the boiling water bath has a marked effect on the color intensity, as shown in Figure 1. The technique of heating at water-boiling temperatures is employed to increase greatly the rate of color development, and since the color intensity varies with the time, the tubes in all of these investigations were heated precisely 10 minutes.

Table II. Cleaning Effects on Blood-Sugar Test Tubes

Test Tube <sup>a</sup>	(0.04 mg. of $\text{Al}_2\text{O}_3$ )		
	Series I	Series II	Series III
11	203	196	190
12	192	188	188
13	169	173	185
14	172	178	187
15	175	180	185
11a	163	170	183
12a	187	186	182
13a	188	188	183
14a	167	169	183
15a	169	170	183

<sup>a</sup> Old test tubes; previous history unknown.

The effect of varying quantities of composite on the color intensity is shown in Figure 2. Since the quantity of composite added influences the color intensity, exactly 10 ml. of the aluminum reagent were added from an automatic pipet.

Since the temperature of the sample and reagents is a factor in this method, a control of  $\pm 5^\circ \text{C.}$  of the solution temperature at the time of standardization should be maintained. Several degrees above and below that at which the curve is established result in no serious error. High temperatures promote color development, with attendant high aluminas, while lower temperatures have the opposite effect.

In the preparation of the composite solution, quantitative and qualitative techniques were applied to several sources of the

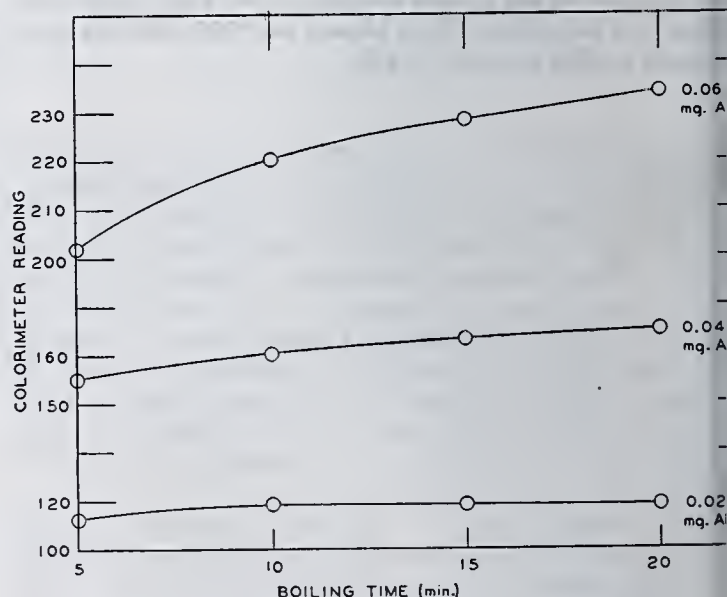


Figure 1. Effect of Heating Time on Color Intensity



gent. To reproduce the values of previously established standardization curves fairly accurately, it became necessary to weigh out the material on a quantitative basis. An Eastman product appeared to give fairly consistent values. Taylor-Austin (12), recognizing the variations in the color produced by the method, suggests a restandardization whenever a fresh supply of the solid reagent is put into use. Scherrer and Smith (11) state that a satisfactory reagent may be synthesized. However, in view of these variabilities and the fact that the composite undergoes a marked change on standing, even when protected from light, the prime necessity of carrying along a standard in routine analysis cannot be overemphasized. It is common practice in this laboratory to establish a new curve when it is observed that the reading of the standard, when compared with the curve, is in error by an amount greater than 4 or 5%. In the desired concentration range, 0.04 mg. of alumina per 25 ml., this represents 6 or 7 scale divisions. For close control work, especially if the curve has not been determined recently, moderate success has been realized in evaluating the scale divisions in the desired concentration range in terms of milligrams of alumina; thus, by running a standard in the concentration range of the unknown, one can calculate the alumina from the colorimeter reading. Obviously, since the milligrams per unit vary over different parts of the curve as well as for different curves, no permanent values should be assessed to each unit.

PRECISION AND ACCURACY

The precision and accuracy of the colorimetric method are conveniently studied by applying the techniques considered in the foregoing paragraphs to the analysis of a leach solution. An initial investigation was concerned with a factor of reproducibility of results. For this purpose two leach liquors, previously acidified and diluted (10 ml. in 250 ml.) were divided into five portions each, and a 1-ml. aliquot (2 ml. in 250 ml.) of each sample was taken for the measurement. The alumina content, recalculated by applying an appropriate factor for dilution (125), was measured over a period of several weeks. Table III, representing ordinary and refined laboratory techniques, shows what might be expected in the way of precision for 10 typically representative

Table III. Analysis of Leach Liquor			
Test No.	Mg./ml.	d	(d <sup>2</sup> × 10 <sup>6</sup> )
Precision of Method under Ordinary Conditions			
1	5.11	-0.063	3969
2	5.23	+0.057	3249
3	5.19	+0.017	289
4	5.23	+0.057	3249
5	5.11	-0.063	3969
6	5.25	+0.077	5929
7	5.14	-0.033	1089
8	5.14	-0.033	1089
9	5.14	-0.033	1089
10	5.19	+0.017	289
Av. = $\bar{X}$	5.173		
$\Sigma d^2 = 24210 \times 10^{-6}$			
$\sqrt{\frac{\Sigma d^2}{10}} = \pm 0.049 = \sigma_{10}$			
$\bar{X} \pm a\sigma = 5.173 \pm 0.053$ ( $P_s = 0.99$ , 10 observations)			
Av. of gravimetric data = 5.114 mg. (Al <sub>2</sub> O <sub>3</sub> ) per ml.			
Precision of Method under Best Conditions			
1	5.11	+0.018	324
2	5.08	-0.012	144
3	5.11	+0.018	324
4	5.04	-0.052	2704
5	5.08	-0.012	144
6	5.08	-0.012	144
7	5.14	+0.048	2304
8	5.07	-0.022	484
9	5.09	-0.002	4
10	5.12	+0.028	784
Av. = $\bar{X}$	5.092		
$\Sigma d^2 = 7369 \times 10^{-6}$			
$\sqrt{\frac{\Sigma d^2}{10}} = \pm 0.027 = \sigma_{10}$			
$\bar{X} \pm a\sigma = 5.092 \pm 0.029$ ( $P_s = 0.99$ , 10 observations)			
Av. of gravimetric data = 5.124 mg. (Al <sub>2</sub> O <sub>3</sub> ) per ml.			

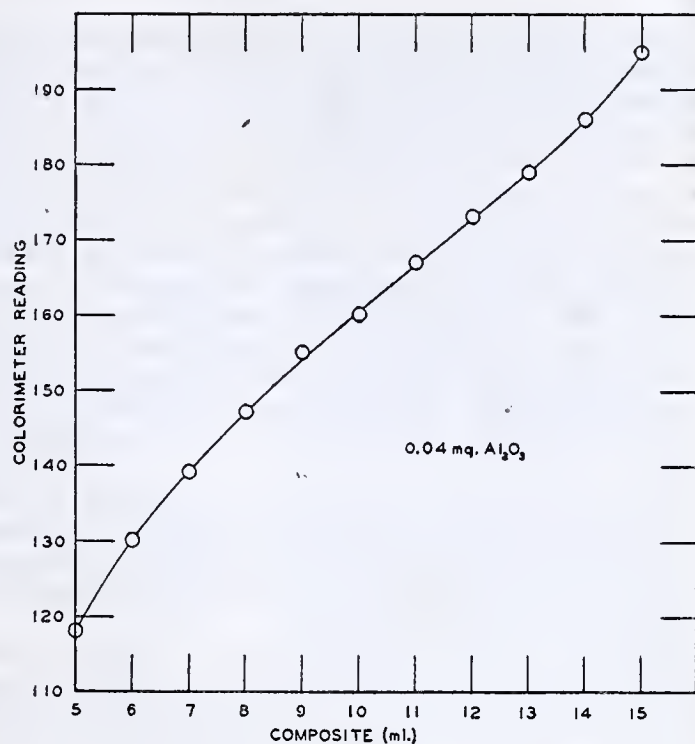


Figure 2. Effect of Varying Quantities of Composite on Color Intensity

values. The method of evaluating the factor of precision is that derived from consideration of results set up by A.S.T.M. (1). As shown in the table, the numerical results are based on the fact that 99 in 100 limits are used and that these results are based completely on the evidence contained in 10 determinations. The accuracy of the method is determined by making a comparison with gravimetric results; gravimetric data for 10 different leach liquors are given in Table IV.

Table IV. Analysis of Leach Liquors				
Accuracy of method under ordinary conditions				
Test No.	Gravimetric Mg./ml.	Colorimetric Mg./ml.	pH	Error %
1	4.113	4.23	6.0	<3
2	3.468	3.43	6.0	1
3	3.640	3.70	6.0	2
4	3.589	3.63	6.0	>1
5	3.637	3.65	6.0	<1
6	3.926	3.93	...	0
7	3.831	3.97	6.0	3
8	3.855	3.82	6.0	1
9	3.732	3.62	6.0	3
10	3.977	3.97	...	0

DISCUSSION

The expression,  $P_s = 0.99$  (statistical probability), cited in Table III implies that a value for  $\alpha$  was chosen such that, in 99 chances out of 100, one might expect the ranges bounded by the computed limits to include, of the universe sampled, the objective average,  $\bar{X}'$ .

From the data in Table III, it is apparent that the colorimetric method should give an average precision, measured by the average deviation of the single results from the mean, of approximately 1%, or 10 parts per 1000. On the basis of the gravimetric value, this represents an accuracy of 1.1%, or 11 parts per 1000. The precision and accuracy are increased by employing refined techniques. In this case special attention was given to temperature control, accurate aliquoting and pipetting, and precise establishment of the standardization curve. However, to attain this precision and accuracy, speed was materially sacrificed. In this case, the average precision becomes 0.6% or 6 parts per 1000. When compared with the gravimetric value, this represents an accuracy of 0.6% or 6 parts per 1000.



The accuracy of the colorimetric method is best judged from the data in Table IV. It is apparent that the accuracy of the method is of the order of 1 to 3%; however, in routine work an occasional 4% error has been observed. In view of the fact that the accuracy is inextricably tied in with the standardization curve, the importance of precise establishment of the calibration curve cannot be overemphasized. If the curve were recently established, then the accuracy and precision become nearly identical if put on the basis of a single analysis. This necessarily follows, since the method involves an empirical comparison against a calibration curve. It is obvious that a higher degree of accuracy will be obtained if, in the preparation of the solution for the colorimetric determination, the concentration of the unknown is approximately adjusted so as to fall in the range above 0.04 mg. per 25 ml.

A further consideration of Table IV reveals the fact that the leach liquors, having been previously acidified with approximately 15 ml. of concentrated hydrochloric acid, when aliquoted to the correct concentration in the presence of excess ammonium acetate, yield a reproducible pH (6.0).

Undoubtedly, colorimetric procedures may be applied for the determination of any element in any given amount by taking suitable aliquot portions for the measurement of the final color; however, applying such a procedure the degree of accuracy will fall markedly as the amount of sample, represented by the aliquots, becomes smaller and smaller. Application of the colorimetric process as a method of analytical control must be decided in terms of the effective range of accuracy by the individual analyst after carefully considering the problem at hand.

#### ACKNOWLEDGMENT

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## Flow Characteristics of Dispersions of Cotton and Regenerated Cellulose Rayon Fabrics in Cuprammonium Their Significance in Fluidity Calculations

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Flow-pressure and fluidity-velocity gradient graphs were used to determine the flow properties of cuprammonium dispersions of cellulose fabrics and to evaluate the method of calculating the fluidity values. The limits of application of the kinetic energy and velocity gradient adjustments were determined for cellulose-cuprammonium dispersions, under experimental conditions very similar to those recommended by the American Society for Testing Materials. The viscometer and buret consistometer were compared in the fluidity technique.

THE fluidity determination of cellulose dispersed in cuprammonium reagent is an important technique used in the study of cotton and regenerated cellulose rayon fabrics. It is a sensitive method for measuring the extent of degradation of cellulose. A linear relationship between fluidity values and service (11, 12) has been demonstrated in serviceability studies of cotton and rayon fabrics which have been subjected to wear and laundering. Raw cotton fibers dispersed in the cuprammonium solvent exhibit a lower fluidity than deteriorated cellulose.

The purpose of this study was to investigate the flow characteristics of cuprammonium dispersions of cotton and regenerated cellulose rayon fabrics over a wide range of fluidity values. The flow properties of cellulose-cuprammonium dispersions heretofore have been studied only in limited scope. It is important to

know which cellulose-cuprammonium dispersions are true viscous liquids and which exhibit anomalous flow properties. This information is fundamental in calculating the fluidities of the liquids.

The flow characteristics of the cellulose-cuprammonium dispersions were determined by means of flow-pressure and fluidity-velocity gradient graphs. As a result of the study of the flow properties of these dispersions, the limits of application of the kinetic energy and velocity gradient adjustments were determined. A comparison was made of the fluidity values obtained with the viscometer and the buret consistometer, and the appropriate instrument for the cellulose-cuprammonium fluidity determination was found to be dependent on the flow characteristics of the dispersion.

#### VISCOMETERS AND METHODS OF CALCULATION OF FLUIDITY VALUES

Two types of capillary tube viscometers are ordinarily used in the fluidity determination of cellulose-cuprammonium dispersions. Figure 1, A, shows the type of viscometer which delivers one or two volumes of liquid, and is recommended by the National Bureau of Standards (10), the American Society for Testing Materials (1), and the British Fabrics Research Committee (8) with attachments described by Conrad (6) and permits the discharge of a number of quantities in one determination. The buret consistometer is used in the Bureaus of Human Nutrition and Home Economics (13) and Agricultural and Industrial Chemistry (6), U. S. Department of Agriculture.

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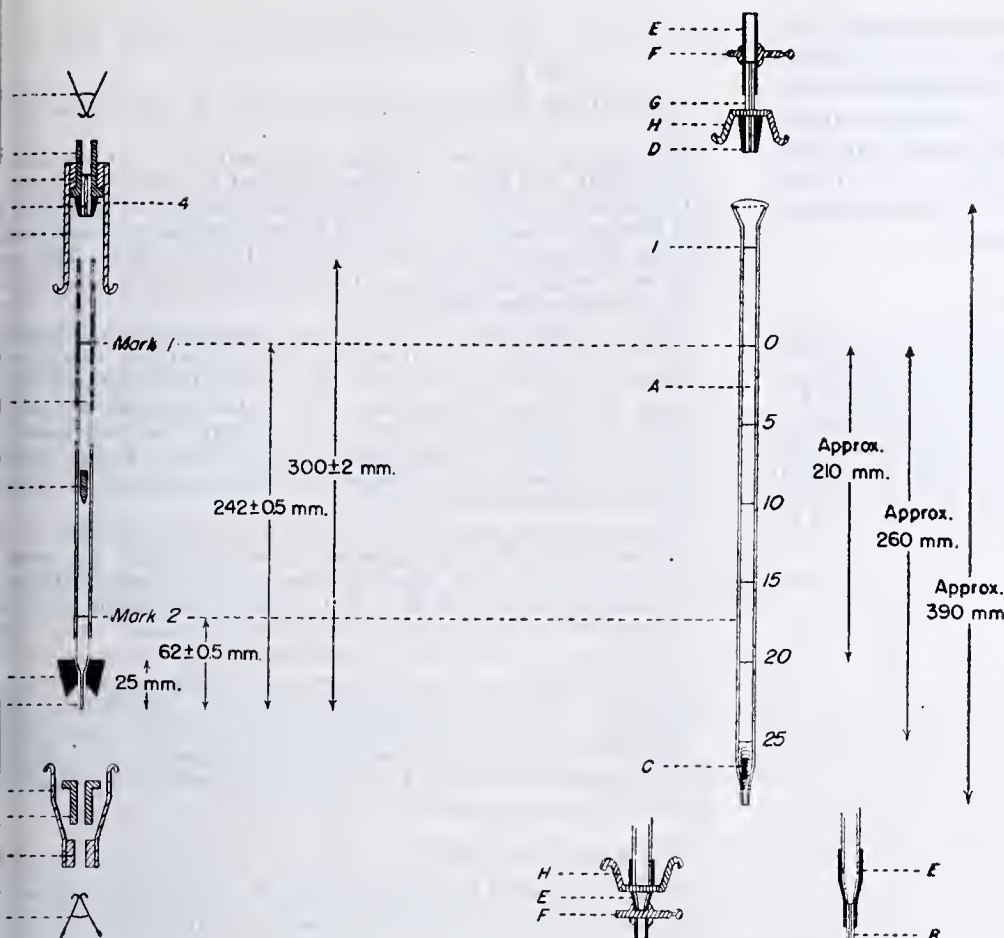


Figure 1. Capillary Tube Viscometers

Left. Single-Volume Viscometer, A

1. Body of viscometer, 10 ± 0.25 mm. inside diameter
2. Capillary discharge tube, 0.88 ± 0.02 mm. inside diameter, 25 ± 0.5 mm. length
3. Steel plunger
4. Rubber stopper
5. Flanged rubber tube
6. Clips
7. Glass capillary
8. Metal hooks for rubber bands
9. Metal collar

Right. Buret Consistometer, B

- A. Body of consistometer, 10 mm. inside diameter
- B. Capillary discharge tube, 0.96 ± 0.02 mm. inside diameter, 25 ± 0.5 mm. length
- C. Steel plunger enclosed in a spring
- D. Rubber stopper
- E. Rubber tube
- F. Screw clamps
- G. Glass capillary
- H. Metal hooks for rubber bands
- I. Mark etched for placement of lower edge of stopper

In the generally accepted procedure of the American Society of Testing Materials (1) and the British Fabrics Research Committee (7) for determining the fluidity of 0.5% solutions of cotton fibers and 2% solutions of regenerated rayon fabrics in cuprammonium, the same method of calculating the fluidity values has been used for all the dispersions regardless of the true viscous or anomalous character of the liquids. These organizations and several investigators (4, 5, 10) use the following formula:

$$F_c = \frac{C'}{t - \frac{K}{t}} \quad \text{or} \quad F_c = \frac{C}{d \left( t - \frac{K}{t} \right)} \quad (1)$$

$F_c$  is the fluidity in rhes corrected for kinetic energy,  $C$  is the instrument constant obtained by calibration (1) with mineral oils of known viscosity,  $C' = \frac{C}{d}$ , in which  $d$  is the density of the cuprammonium solvent in grams per milliliter,  $t$  is the time of flow in seconds, and  $K$  is the kinetic energy correction constant obtained by calibration (1, 5). It is convenient to consider  $d$  constant since the density of cuprammonium suspensions do not differ essentially from the density of the cuprammonium solvent. Formula 1 is a convenient way of expressing the reciprocal of the well-known Poiseuille equation.  $C$  in Equation 1 is equal to  $\frac{LV}{8\pi r^4 h}$  in the Poiseuille equation, and  $K$  equals  $\frac{mVC}{8\pi L}$ . The Poiseuille equation and Equation 1 are applicable only to the streamline flow of true viscous liquids.

Since many cellulose-cuprammonium dispersions are not true viscous liquids, and the Poiseuille equation is not strictly appli-

cable and gives so-called "apparent fluidities", Downey and Elmquist (13) adapted the following equation to the calculation of the fluidities of dispersions of cellulose fabrics in cuprammonium:

$$F_0 = \frac{q}{C(P - p)} \quad (2)$$

$F_0$  in rhes is Bingham's (3) mobility value which is not corrected for kinetic energy,  $q$  is the rate of flow in milliliters per second,  $C$  is the instrument constant obtained by calibration with mineral oils of known viscosity,  $P$  is the hydrostatic pressure in grams per square centimeter, and  $p$  is the yield value. The yield value is the minimum pressure required to establish the flow of a plastic material, and is graphically determined as the intercept of the graph prolonged on the axis of the abscissa when the hydrostatic pressure is plotted as abscissa and the rate of flow,  $q$ , as ordinate (Figure 2). When the dispersion is a true viscous liquid the yield value,  $p$ , is 0, and Equation 2 becomes  $F_0 = \frac{q}{CP}$ , which in terms of the Poi-

seuille equation is  $F_0 = \frac{8LV}{\pi g r^4 h d l}$  for  $C = \frac{\pi g r^4}{8L}$ ,  $P = hd$ , and  $q = \frac{V}{t}$ .

Equation 2 is suitable for calculating the mobility of plastic materials. It would be satisfactory for calculating the fluidity values of cellulose-cuprammonium dispersions if they were plastic liquids having real yield values and flowing at such a slow rate that the kinetic energy correction is not necessary.

The most recent contribution to the calculation of the fluidity of cellulose-cuprammonium dispersions was

made by Conrad (6), who adjusted the fluidity observed at different mean velocity gradients to a standard or reference velocity gradient.

When a liquid flows through a capillary tube, the central part of the column of liquid flows most rapidly while the liquid in contact with the capillary wall is nearly stationary, and the layers of the liquid column slip past each other between those two limits. The velocity gradient is the rate of change in the velocity of the adjacent layers of the liquid per unit of distance measured at right angles to the direction of the velocity. In the capillary flow method the viscosity or fluidity of a true liquid is independent of the velocity gradient, but the fluidity of an anomalous liquid depends on the velocity gradient and has an indefinite number of values at different points in the cross section of the capillary tube.

Conrad (6) calculated the mean velocity gradient in capillary tubes by the equation:

$$G = \frac{8V}{3\pi r^3 t} \quad (3)$$

which was derived by Kroepelin (9) in 1929.  $G$  is the mean velocity gradient in centimeters per second per centimeter and  $V$  is the volume in milliliters of the solution discharged in  $t$  seconds through a capillary of radius  $r$  centimeters. Conrad recognized that  $G$ , calculated from Equation 3, is an approximation rather than a true value for anomalous dispersions of cellulose in cuprammonium solvent.

The velocity gradient adjustment was shown by Conrad (6) to eliminate the variation of the fluidity values of highly anoma-



lous cellulose-cuprammonium dispersions determined at different average pressures in the buret consistometer. He also pointed out that the velocity gradient adjustment overcomes differences in fluidity values between instruments in the cellulose-cuprammonium fluidity determination. However, he studied dispersions in the very limited fluidity range of approximately 2 to 3 rhes. The scope of the anomalous behavior of cellulose-cuprammonium dispersions has not been determined heretofore.

#### EXPERIMENTAL PROCEDURE

The buret consistometer with dimensions and parts as illustrated in Figure 1, *B*, was used in the fluidity determinations. The consistometers were calibrated according to the method of Herschel and Bulkley (8) with mineral oils of known viscosity obtained from the National Bureau of Standards. The value of  $m$ , the coefficient of kinetic energy correction for which the constant value 1.12 is ordinarily assumed, was calculated for each of thirty consistometers (8) and was found to vary from 1.04 to 1.32. The inside capillary diameters of thirty instruments were calculated according to Herschel and Bulkley (8) and were found to vary between the limits of 0.0942 to 0.0985 cm. Constants  $C'$  and  $K$  in Equation 1 were calculated according to the directions of the American Society for Testing Materials (1) and Clibbens and Little (5). The hydrostatic pressure,  $P$ , in the flow-pressure graphs, was calculated by the equation  $P = hd$ . The average head,  $h$ , was obtained with Meissner's for-

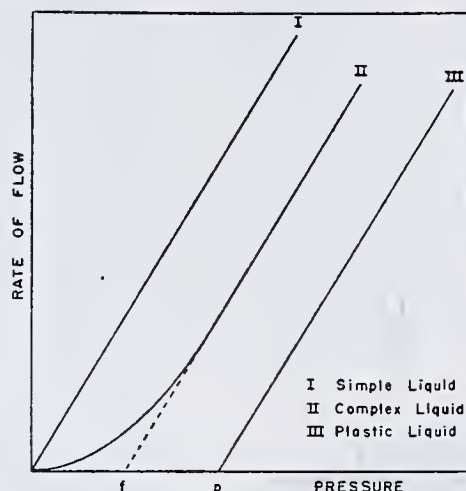


Figure 2. Flow-Pressure Graphs of Simple, Complex, and Plastic Liquids

mula,  $h = \frac{h_1 - h_2}{\log_e \frac{h_1}{h_2}}$ , and was corrected for surface tension (8, 1, 7).

The density of the cuprammonium solvent,  $d$ , has a value of 0. (1, 7).

The cuprammonium solution contained  $15 \pm 0.1$  grams copper,  $200 \pm 5$  grams of ammonia, and less than 0.5 gram nitrites per liter, which are the specifications of the British Farries Research Committee (7) and the more accurate limits stated by the American Society for Testing Materials (1). The solution was prepared, stored, and delivered with equipment similar to that designed by Mease (10).

The concentrations of the cellulose-cuprammonium dispersions were 0.5 and 2.0% by volume for the cotton and regenerated cellulose rayon fabrics, respectively. Forty-two cuprammonium rayon, 165 viscose rayon, and 773 cotton samples at various stages of degradation were used to investigate the flow characteristics of cellulose-cuprammonium dispersions. These samples had been subjected to ordinary household wear and laundering without drastic chemical treatment.

The procedure employed for determining the fluidity of the cellulose-cuprammonium dispersions was essentially the same as that described by the American Society for Testing Materials (1), except the rate of flow of the dispersions from the buret consistometer was obtained with a "split second" stop watch in two ways in the same determination: as a series of observations corresponding to the successive 5-ml. quantities, and as the rate of flow of a single 20-ml. discharge. The 20-ml. volume rather than the 25-ml. quantity was used because it more nearly corresponds to the volume between calibration marks 1 and 2 of the viscometer (Figure 1, 4). The temperature during conditioning and timing was controlled at  $20^\circ \pm 0.1^\circ \text{C}$ .

One fluidity determination with a buret consistometer furnishes sufficient data for a flow-pressure graph. The single-volume viscometer does not provide data for such a graph unless determinations are repeated at different pressures, which would require additional apparatus.

#### STUDY OF FLUIDITY DATA

FLOW-PRESSURE RELATIONSHIPS OF REGENERATED CELLULOSE RAYON FABRICS IN CUPRAMMONIUM. Flow-pressure graphs

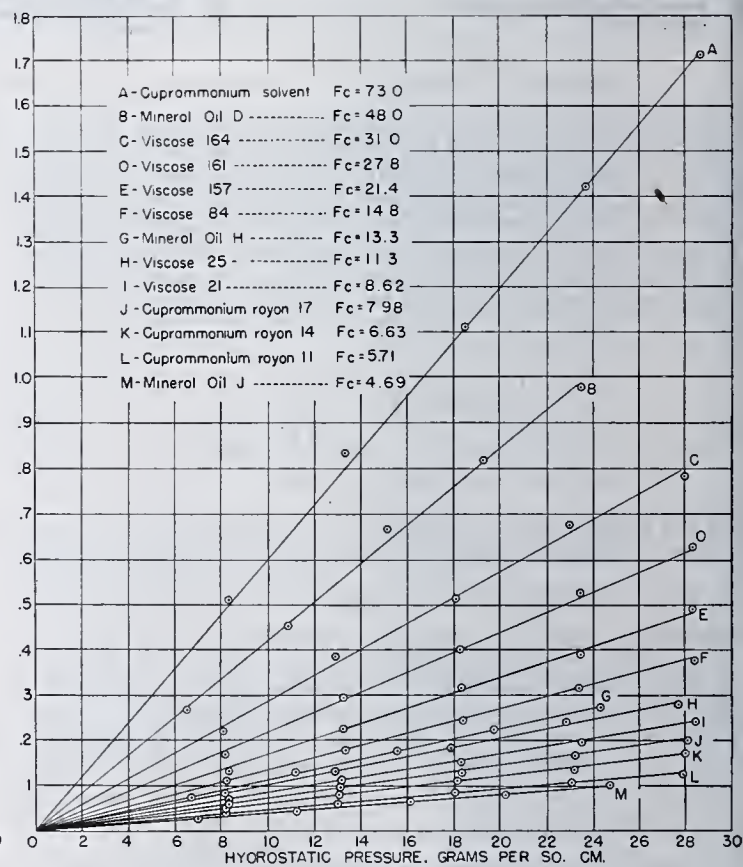
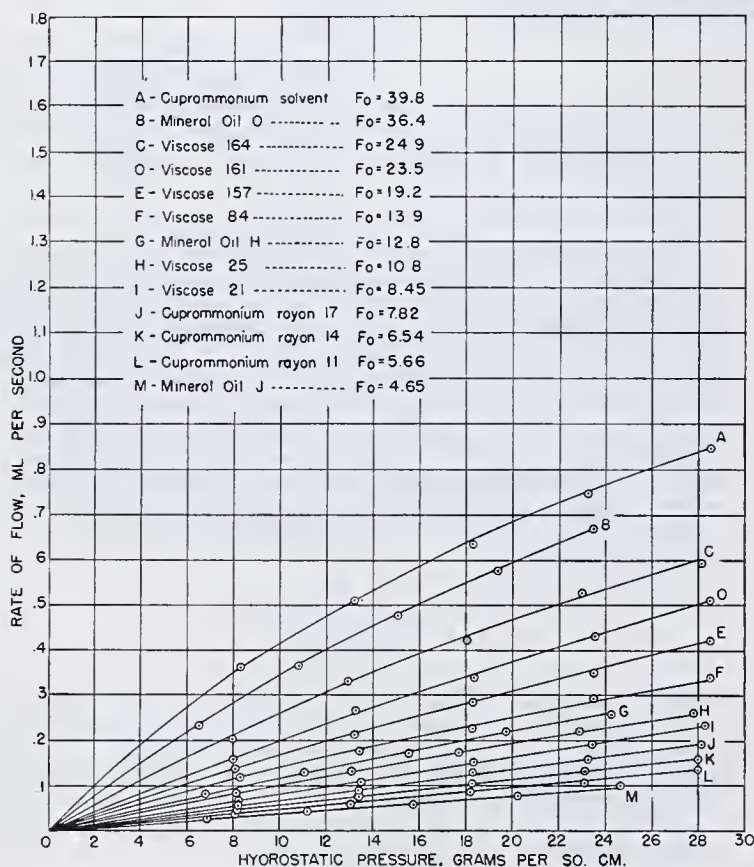


Figure 3. Flow-Pressure Graphs of Simple Liquids

A (left), observed flow values. B (right), flow values corrected for kinetic energy.



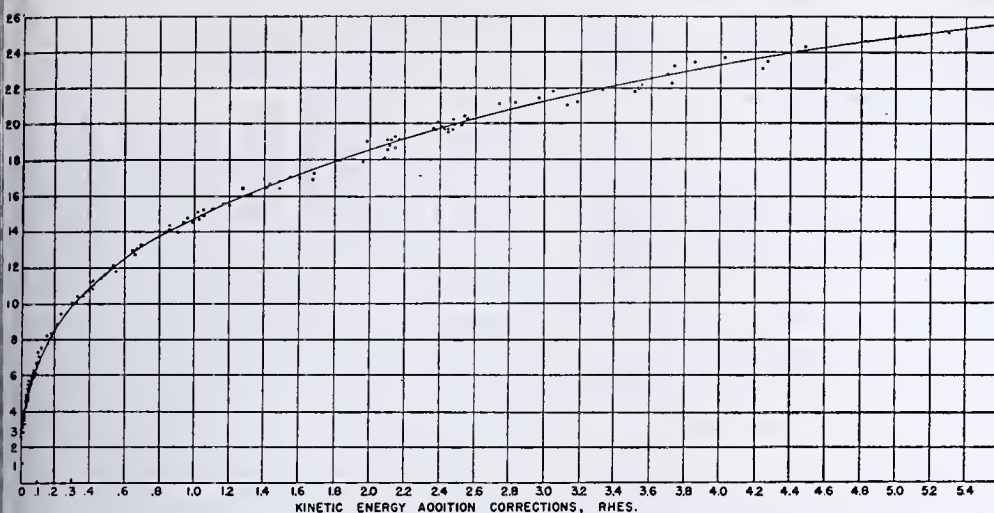


Figure 4. Magnitude of Kinetic Energy Correction

Calculation of fluidity of cellulose fabrics dispersed in cuprammonium, using capillaries with inside diameters of from 0.094 to 0.098 cm.

which the average hydrostatic pressure,  $P$ , is plotted as abscissa and the rate of flow,  $q$ , as ordinate, indicate the flow properties of a liquid. It is well known that the flow-pressure graph of a simple liquid, also called a Newtonian or true viscous liquid, is a straight line passing through the origin—for example, I in Figure 2. The American Society for Testing Materials (2) defines a simple liquid as one in which the rate of shear is proportional to the rate of stress, and a complex liquid as one in which the rate of shear is not proportional to the shearing stress. The flow-pressure graph of a complex liquid, II in Figure 2, passes through the origin but is not linear. Complex liquids have also been called pseudoplastic (14). A complex liquid does not have a real yield value; the apparent yield value,  $f$  in Figure 2, is obtained by disregarding the lower curved part of the graph. III in Figure 2 is the flow-pressure graph of a plastic material, a straight line which does not pass through the origin. A plastic material has a real yield value,  $p$  in Figure 2, which is the minimum pressure required to establish flow.

In Figure 3 the flow-pressure graphs of cuprammonium dispersions of cuprammonium and viscose rayon are compared with those of known simple liquids—that is, mineral oils  $J$ ,  $H$ , and  $D$ , and cuprammonium solvent. Each fluidity value given for a cellulose dispersion is the average of the 4 values obtained from a single discharge with the buret consistometer. The rates of flow in Figure 3,  $A$ , are uncorrected; those in Figure 3,  $B$ , are corrected for kinetic energy.

In the uncorrected flow-pressure graphs, the liquids with a slow rate of flow—for example, mineral oil  $J$  and cuprammonium rayon samples 11, 14, and 17—are represented by linear graphs. For these liquids the kinetic energy correction is relatively unimportant. As the rate of flow increases and the kinetic energy correction becomes more important, the uncorrected curves become increasingly concave to the pressure axis. When the data are corrected for kinetic energy, the curvature of the flow-pressure graphs was removed, and all the graphs in Figure 3,  $B$ , came linear, passing through the origin. Forty-two cuprammonium rayon samples ranging in corrected fluidity value,  $F_c$ , from 4.52 to 8.26 rhes were found to form the typical flow-pressure graphs of simple liquids. The characteristic flow-pressure graphs of three representative cuprammonium rayon dispersions are included in Figure 3.

The flow-pressure graphs of 165 viscose rayon dispersions ranging in  $F_c$  values from 7.51 to 32.9 rhes were those of simple liquids. The fluidity of the six representative viscose dispersions in Figure 3 increases, the curvature of the uncorrected graphs becomes more evident.

**KINETIC ENERGY CORRECTION.** The importance of the kinetic energy correction is apparent in the comparison of the graphs in Figure 3. In a fluidity determination the kinetic energy correction varies continuously as the dispersion flows from the

tube, and decreases as the flow becomes slower. The effect of the kinetic energy correction on the fluidity calculations when the buret consistometer is used is shown in Table I with representative data for the simple rayon dispersions. The uncorrected fluidity values,  $F_0$ , of the dispersions of higher fluidity, viscose 64, 156, and 164, increase in value as the rate of flow decreases in the discharge from the consistometer. When corrected for kinetic energy, the corresponding values,  $F_c$ , in Table I become constant within experimental error.

The magnitude of the kinetic energy correction was calculated over the entire range of fluidities of cellulose fabric dispersions using capillary discharge tubes with inside diameters varying from 0.094 to 0.098 cm., a specification nearly the same as 0.088 = 0.002 cm. inside diameter recommended by the American Society for Testing Materials (1). In Figure 4 the average of the uncorrected fluidity values of representative cuprammonium rayon, viscose rayon, and cotton samples obtained with the consistometer is plotted as ordinate and the average kinetic energy correction as abscissa. This graph shows that the uncorrected fluidity values below 7 rhes have a kinetic energy correction of not more than 0.1 rhe, a negligible error of approximately 1% or less. Thus, for fluidities less than 7 rhes the equation,  $F_0 = \frac{C'}{t}$  (Equation 4), in which  $F_0$  is not corrected for kinetic energy, may be used.

In the range of uncorrected fluidities from 8 to 13 rhes, the magnitude of the kinetic energy correction varies from approximately 2 to 5%, and whether Equation 1 or 4 is used for the calculation depends on the accuracy required. For uncorrected fluidity values of more than 13 rhes, the kinetic energy correction becomes greater than 5%, increasing markedly as the fluidity increases, and Equation 1 is suitable. Clibbens and Geake (4) recognized that the kinetic energy correction is important in cellulose-cuprammonium fluidity calculations and stated that it may amount to 20 to 30% in the case of highly modified cellulose samples.

**FLOW-PRESSURE RELATIONSHIP OF COTTON FABRICS DISPERSED IN CUPRAMMONIUM.** The series of flow-pressure graphs of the dispersions of cotton fabrics in cuprammonium which are plotted in Figure 5 are representative of the graphs of the 773

Table I. Observed Fluidity Values, Fluidity Values Corrected for Kinetic Energy, and Kinetic Energy Correction for Four Rayon Fabrics

Sample	Consistometer Ring Interval Ml.	Observed Fluidity <sup>a</sup> Rhes	Fluidity Corrected for Kinetic Energy <sup>b</sup> Rhes	Kinetic Energy Correction Rhes
Cuprammonium rayon 6	0-5	4.93	4.97	0.04
	5-10	4.90	4.94	0.04
	10-15	4.94	4.97	0.03
	15-20	4.98	5.00	0.02
Viscose rayon 64	0-5	12.02	12.69	0.67
	5-10	12.16	12.74	0.58
	10-15	12.26	12.71	0.45
	15-20	12.38	12.71	0.33
Viscose rayon 156	0-5	16.05	17.69	1.64
	5-10	16.39	17.92	1.53
	10-15	16.71	17.94	1.23
	15-20	16.82	17.71	0.89
Viscose rayon 164	0-5	22.96	30.14	7.18
	5-10	24.72	31.64	6.92
	10-15	25.60	31.35	5.75
	15-20	26.47	31.05	4.58

<sup>a</sup> Observed fluidity values calculated by equation,  $F_0 = \frac{C'}{t}$ .

<sup>b</sup> Fluidity values corrected for kinetic energy calculated by equation,  $F_c = \frac{C'}{t - \frac{K}{t}}$ .



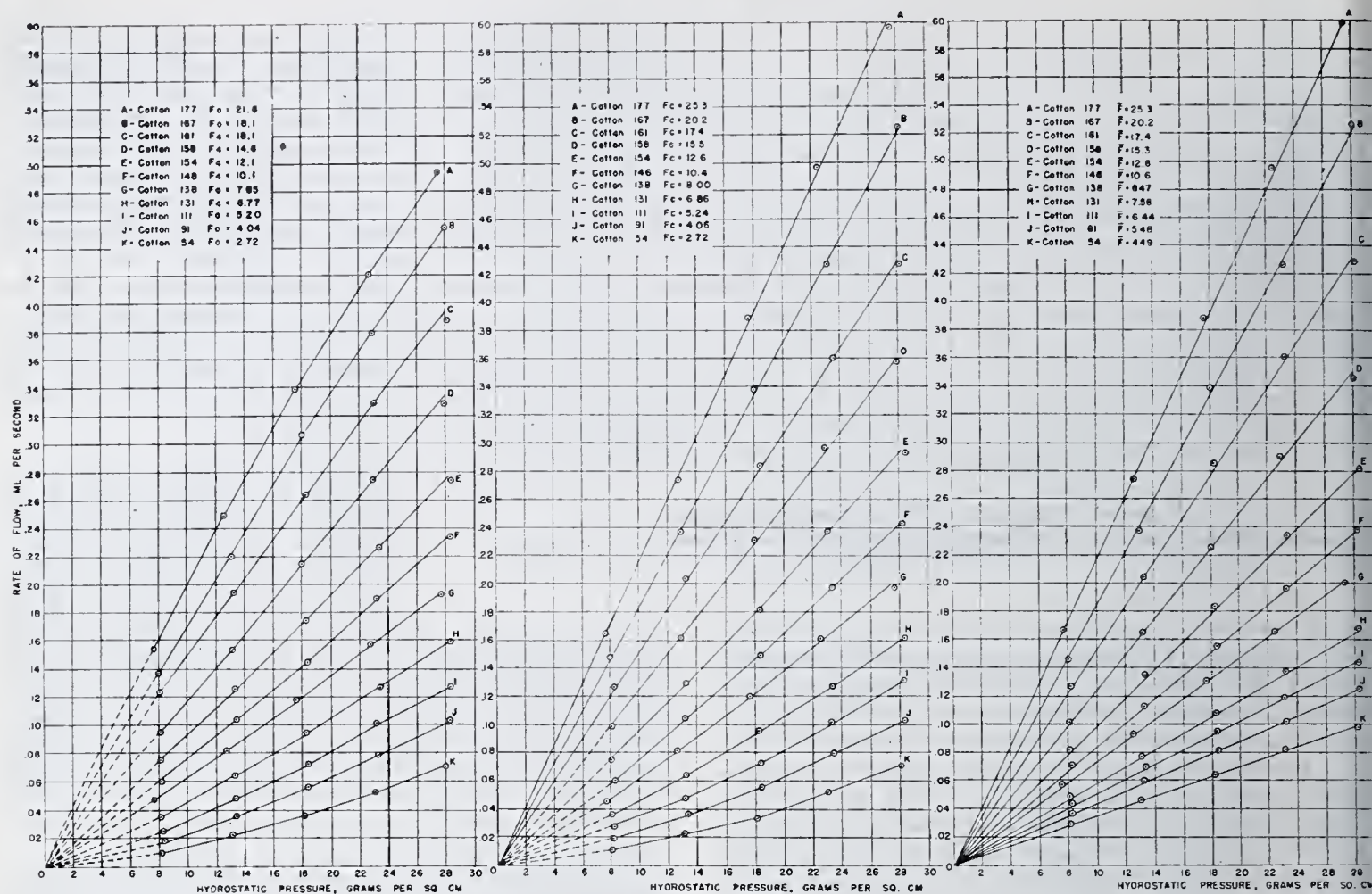


Figure 5. Flow-Pressure Graphs of Cotton Fabrics Dispersed in Cuprammonium

A (left), uncorrected flow values. B (center), flow values corrected for kinetic energy. C (right), flow values adjusted for kinetic energy and velocity gradient

cotton dispersions studied. Each fluidity value is the average of the 4 values obtained in a single discharge with the buret consistometer.

The curvature of the uncorrected flow-pressure graphs of the more rapidly flowing dispersions of cottons 161, 167, and 177 is concave to the pressure axis (Figure 5, A). When the rate of flow is corrected for kinetic energy, the graphs for these cottons become linear and pass through the origin (Figure 5, B). Thus, the cuprammonium dispersions of cottons 161, 167, and 177 are those of simple liquids, the Poiseuille equation is applicable, and the fluidity values should not be described as "apparent".

The uncorrected curves in Figure 5, A, of the cotton dispersions of lower fluidity, cottons 54 to 158, are convex to the pressure axis, the curvature decreasing with increase in fluidity. This convex curvature is not removed by applying the kinetic energy correction (Figure 5, B). The graphs of these dispersions of cottons varying in  $F_c$  values from 2.72 to 15.5 rhes are those of complex or pseudoplastic liquids (Figure 2). They do not form the curves of plastic materials; and the terms "plasticity" or "plastic behavior" should not be used to describe the flow properties of these 0.5% cuprammonium dispersions of cotton fabrics.

When the kinetic energy and the velocity gradient adjustments are both applied to the rate of flow of the complex cotton-cuprammonium dispersion 54 to 158, the convex curvature is removed and the flow-pressure graphs all become linear and pass through the origin (Figure 5, C). The velocity gradient adjustment does not influence the flow-pressure graphs of the dispersions of cotton 161, 167, and 177 (Figure 5, C) since they are simple liquids.

**VELOCITY GRADIENT ADJUSTMENT.** The effect of the velocity gradient adjustment on the fluidity values of dispersions of cellulose fabrics is shown with some representative examples in Table II. The  $F_c$  values were calculated with

Equation 1, and the  $G$  values, mean velocity gradients, with Equation 3. The  $\bar{F}$  values are the fluidities adjusted both for kinetic energy and velocity gradient.

The average  $\bar{F}$  value may be read directly from a fluidity-velocity gradient graph (explained below, see Figure 8) by interpolation or extrapolation where the sloping line crosses the vertical standard velocity gradient line. However, if the  $\bar{F}$  values corresponding to the successive 5-ml. volumes delivered by the buret consistometer are required, as in Table II, they are calculated from Equation 5:

$$\log \bar{F} - \log F_c = \text{regression coefficient} \times (\log \bar{G} - \log G)$$

This is the well-known equation of a straight line,  $y - y_1 = m(x - x_1)$ , where  $m$  is the slope of the line which passes through

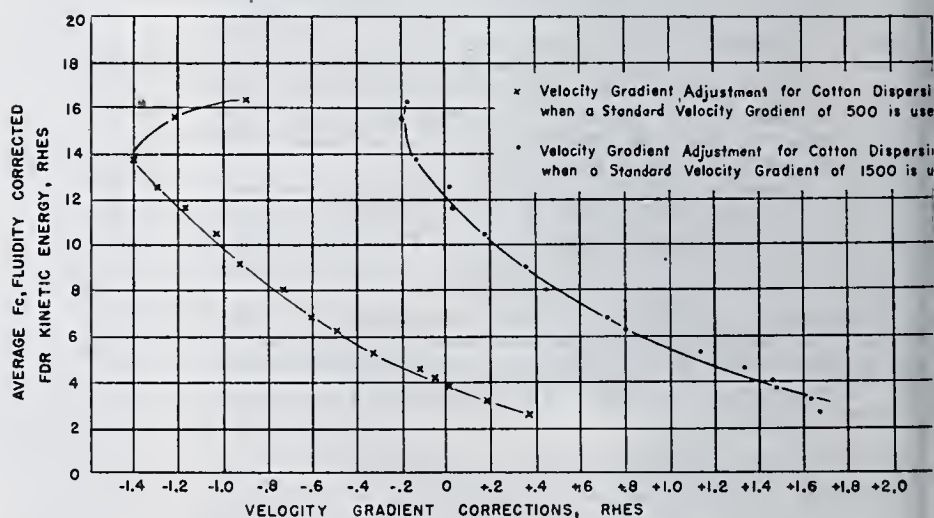


Figure 6. Comparison of Magnitude of Velocity Gradient Adjustment



point  $x_1, y_1$ . The regression coefficient, the slope of the line, is obtained graphically.  $G$  is the mean velocity gradient calculated with equation 3 and  $\bar{G}$  is the standard velocity gradient.

Table II, A and B, shows that  $F_c$  values obtained with a buret consistometer for the complex cotton dispersions 54, 111, 138, and 158 increase with a decrease in mean velocity gradient as the liquid flows out of the consistometer and the pressure head becomes smaller. If the values are calculated to a standard or reference velocity gradient, the values are corrected for the variation caused by the change in the velocity gradient. When the fluidity of the complex cotton-cuprammonium dispersions are calculated to the standard velocity gradient of 1500, corrected fluidity values,  $\bar{F}_{1500}$ , become constant within experimental error (Table II, C). The standard velocity gradient is entirely arbitrary and it would appear from Figure 8 at almost any intermediate velocity gradient could be selected. Conrad (6) recommended the use of a standard velocity gradient of 500 cm. per second per cm., "since a large part of the data recorded in the literature has been obtained probably at velocity gradients between 100 and 1000 cm. per second per cm., a convenient value might be intermediate, say at 500 cm. per second per cm." Under the conditions of this study, which are recommended by the American Society of Testing Materials (1), the maximum average velocity gradient is approximately 3000 cm. per second per cm. for complex 0.5% cotton-cuprammonium dispersions with  $F_c$  values less than 17 rhes (Figure 8). Thus a common velocity gradient of 1500 is suggested, since it is intermediate for these complex dispersions.

When the average  $F_c$  values of Figure 8 are plotted against the velocity gradient adjustment, Figure 6, a standard velocity gradient of 1500 gives an adjustment curve which nearly approaches zero tangentially. As the  $F_c$  values increase to those characteristic of simple liquids. At some velocity gradient slightly above 1500, a curve corresponding to that in Figure 6 would more nearly approach zero. If a standard velocity gradient of 500 had been used, an anomalous dispersion with a  $F_c$  value of approximately 4 rhes would have a zero velocity gradient adjustment

**Table II. Comparison of Fluidity Values Corrected for Kinetic Energy ( $F_c$ ) with Fluidity Values Adjusted for Both Kinetic Energy and Velocity Gradient Using the Standard Velocity Gradient of 1500 ( $\bar{F}_{1500}$ )**

Type of Dispersion	Sample	Buret Consistometer Values from Consecutive 5-Ml. Volumes				Single-Volume Viscometer Values, Varying Placement of Lower Mark			
		A		B		D		E	
		Consistometer ring interval	Mean velocity gradient	$F_c$	$\bar{F}_{1500}$	Volume	Mean velocity gradient	$F_c$	$\bar{F}_{1500}$
		Ml.	Cm./sec./cm.	Rhes	Rhes	Ml.	Cm./sec./cm.	Rhes	Rhes
Complex cotton-cuprammonium dispersions	Cotton 54	0-5	566	3.20	4.46	0-5	566	3.20	4.50
		5-10	427	2.94	4.51	0-10	487	3.05	4.52
		10-15	288	2.55	4.47	0-15	396	2.82	4.49
		15-20	179	2.20	4.53	0-20	304	2.56	4.47
			Av.	2.72	4.49		Av.	2.91	4.50
	Cotton 111	0-5	1015	5.84	6.44	0-5	1015	5.84	6.49
		5-10	800	5.48	6.41	0-10	895	5.63	6.47
		10-15	575	5.05	6.42	0-15	755	5.38	6.47
		15-20	384	4.61	6.48	0-20	608	5.07	6.47
			Av.	5.24	6.44		Av.	5.48	6.48
	Cotton 138	0-5	1435	8.41	8.46	0-5	1435	8.41	8.46
		5-10	1169	8.20	8.47	0-10	1288	8.12	8.48
		10-15	876	7.91	8.48	0-15	1114	8.29	8.49
		15-20	601	7.48	8.42	0-20	918	7.88	8.48
			Av.	8.00	8.46		Av.	8.18	8.48
	Cotton 158	0-5	2548	15.89	15.36	0-5	2548	15.89	15.27
		5-10	2139	15.61	15.26	0-10	2326	15.74	15.23
		10-15	1655	15.45	15.35	0-15	2049	15.62	15.26
		15-20	1203	15.12	15.33	0-20	1742	15.43	15.26
			Av.	15.52	15.32		Av.	15.66	15.26
Simple cellulose-cuprammonium dispersions	Cuprammonium rayon 6	0-5	859	4.97	4.97	0-5	859	4.97	4.97
		5-10	711	4.94	4.94	0-10	778	4.95	4.95
		10-15	553	4.97	4.97	0-15	685	4.96	4.96
		15-20	396	5.00	5.00	0-20	579	4.98	4.98
			Av.	4.97	4.97		Av.	4.96	4.96
	Viscose rayon 25	0-5	1907	11.34	11.34	0-5	1907	11.34	11.34
		5-10	1615	11.27	11.27	0-10	1749	11.28	11.28
		10-15	1271	11.28	11.28	0-15	1554	11.31	11.31
		15-20	917	11.13	11.13	0-20	1324	11.23	11.23
			Av.	11.26	11.26		Av.	11.29	11.29
	Cotton 177	0-5	3680	25.38	25.38	0-5	3680	25.43	25.43
		5-10	3123	25.30	25.30	0-10	3379	25.31	25.31
		10-15	2545	26.08	26.08	0-15	3046	25.63	25.63
		15-20	1840	25.02	25.02	0-20	2617	25.41	25.41
			Av.	25.44	25.44		Av.	25.44	25.44

(Figure 6) which is characteristic only of simple dispersions. Thus, in this study, the standard velocity gradient of 1500 was preferred.

According to Figure 6 the velocity gradient correction to a common velocity gradient of 1500 is not necessary if  $F_c$  is greater than 10 rhes.

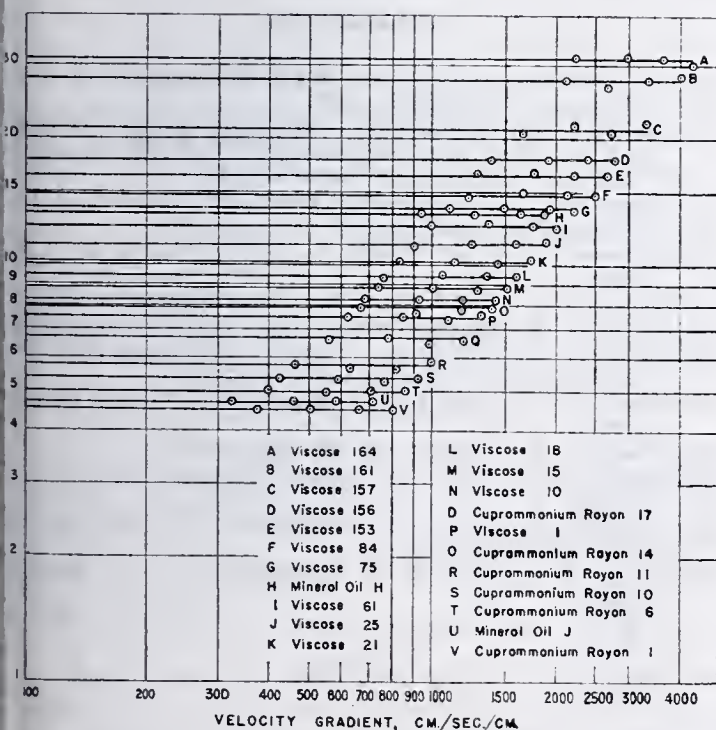
The fluidity values of the complex cotton fabric dispersions obtained from the two types of capillary instruments are not the same unless the velocity gradient adjustment is applied (Table II, B and E). Using the consistometer as a single-volume viscometer, the  $F_c$  values of the complex cotton dispersions 54, 111, 138, and 158 vary with the position of the lower mark, decreasing as the viscometer volume increases (Table II, E). However, when the velocity gradient adjustment is applied, the fluidity values become constant, regardless of where the mark is placed in the single-volume viscometer (Table II, F).

Also, until the velocity gradient adjustment is applied, the average of the four values obtained with the buret consistometer from the discharge of 20 ml. of liquid is not equal to the fluidity value calculated from the rate of flow of a single 20-ml. quantity discharged from the same instrument when used as a viscometer (Table II).

Since the velocity gradient adjustment is readily obtained with the buret consistometer in one discharge, the buret type of capillary instrument has an advantage over the single-volume viscometer type for the determination of the fluidity of complex cotton-cuprammonium dispersions with  $F_c$  values less than 10 rhes.

In the fluidity calculations of the cellulose-cuprammonium dispersions which are simple liquids—for example, cuprammonium rayon 6, viscose 25, and cotton 177 in Table II—the fluidity values are constant with the application of the kinetic energy correction; and the fluidity is not influenced by the velocity gradient.

The fluidity values for the simple cellulose-cuprammonium dispersions obtained with the two kinds of instruments are the same for any viscometer volume or part of the buret consistometer. Thus either the buret consistometer or the viscometer may be used for these simple dispersions.



**Figure 7. Fluidity-Velocity Gradient of Viscose and Cuprammonium Rayon Fabrics Dispersed in Cuprammonium**



**FLUIDITY-VELOCITY GRADIENT RELATIONSHIPS.** Fluidity-velocity gradient graphs as well as flow-pressure graphs indicate the simple or complex character of a liquid. The former are plotted on log-log paper with the mean velocity gradient, computed with Equation 3, as abscissa and the corresponding  $F_c$  value obtained with the buret consistometer as ordinate.

The fluidity-velocity gradient graph of a simple liquid, such as mineral oils *J* and *H* in Figure 7, is a horizontal straight line, because the fluidity of a simple liquid is independent of the velocity gradient. The fluidity-velocity gradient graphs of Figure 7 show that 2% cuprammonium rayon dispersions ranging from  $F_c$  values of 4.52 to 7.98 rhes are simple liquids. Likewise, 2% viscose dispersions with  $F_c$  values from 7.51 to 31.0 are all simple liquids. Thus the same information concerning the flow properties of the regenerated rayon-cuprammonium dispersions is obtained from both the flow-pressure (Figure 3) and the fluidity-velocity gradient graphs.

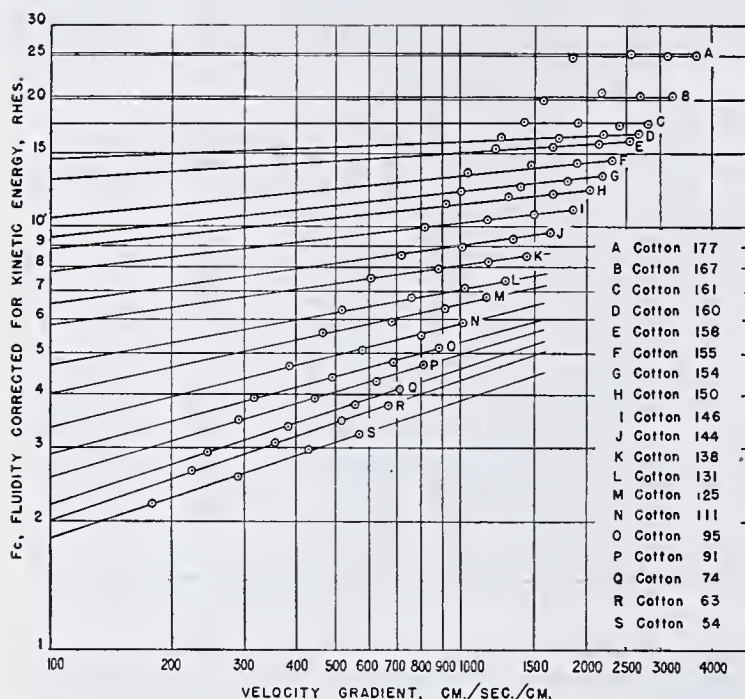


Figure 8. Fluidity-Velocity Gradient of Cotton Fabrics Dispersed in Cuprammonium

The fluidity-velocity gradient graphs of the complex cuprammonium dispersions of cottons 54 to 160 in Figure 8 are linear and sloping. The velocity gradient values of complex cotton-cuprammonium dispersions increase as their fluidities increase. In proceeding from the graphs of cotton 54 to 160, the slopes decrease and approach zero, and the dispersions become less complex. The fluidity-velocity gradient graphs of cottons 161, 167, and 177 are linear and horizontal, those of simple liquids, which is in agreement with the information obtained from the flow-pressure graphs of Figure 5.

It is apparent from Figure 8 that under the experimental conditions the cotton-cuprammonium dispersions with fluidities of approximately 17 rhes are borderline between simple and complex liquids. The fluidity-velocity gradient graphs of 61 cotton dispersions varying in  $F_c$  from 16 to 21 rhes were examined in order to determine the limits of the borderline values. The graphs of 12 dispersions varying in  $F_c$  values from 16.0 to 17.1 rhes were definitely sloping. Twenty-nine graphs of dispersions with  $F_c$  values between 17.2 and 18.7 rhes were variable; of these, nine graphs sloped, five were horizontal, and 15 could have been considered either horizontal or slightly sloping. Twenty graphs with  $F_c$  values from 18.9 to 21.0 rhes were unquestionably horizontal. Thus 0.5% cotton-cuprammonium dispersions with  $F_c$  values less than 17 rhes are complex liquids, and those with  $F_c$  values greater than 19 rhes are simple liquids. The cases where the  $F_c$  values are between 17.0 and 18.9 rhes are borderline.

These observations on the range of complex flow of 0.5% cotton-cuprammonium dispersions agree with tests by Clibbens and Little (5) of the deviations of cotton cuprammonium dispersions from the Poiseuille law by means of a time ratio,  $t_1/t_2$ . In this ratio  $t_1$  is the time in seconds for the first volume of liquid to flow from a two-volume viscometer, and  $t_2$  the time for the second

quantity. The  $t_1/t_2$  ratio for a true viscous liquid was equal to unity, provided the kinetic energy correction was small; the time ratio for an anomalous liquid was less than unity. For an  $F_c$  value of 15.0 rhes, they reported  $t_1/t_2$  equal to 0.98 and for an  $F_c$  value of 21.3,  $t_1/t_2$  was 1.00. They gave no values between 1 and 21.3 rhes.

## SUMMARY AND CONCLUSIONS

The flow characteristics of cotton and regenerated cellulose fabrics dispersed in cuprammonium were determined by means of flow-pressure and fluidity-velocity gradient graphs. Under the experimental conditions recommended by the American Society for Testing Materials, 0.5% cotton-cuprammonium dispersion with fluidity values ( $F_c$  corrected for kinetic energy) greater than 19 rhes and 2% regenerated cellulose dispersions are simple liquids; 0.5% cotton-cuprammonium dispersions with  $F_c$  values less than 17 rhes are complex liquids rather than plastic materials, since they do not exhibit a real yield value.

The formula,  $F_0 = \frac{C'}{t}$ , may be used for the calculation of fluidity values less than 7 rhes, since the kinetic energy correction for these values is negligible. Either the Poiseuille equation or Equation 1,  $F_c = \frac{C'}{t - \frac{K}{t}}$ , more frequently used in calculating the

fluidity of cellulose dispersions, is suitable for calculating fluidity values greater than 7 rhes. The velocity gradient adjustment is applicable to the complex cotton-cuprammonium dispersion with  $F_c$  values less than 10 rhes. Equation 2, intended for plastic materials, should not be used for calculating the fluidities of cellulose fabrics since they form either simple or complex rather than plastic dispersions, and they often require the kinetic energy correction.

Either the buret consistometer or the viscometer may be used for the regenerated cellulose dispersions or the cotton dispersions with  $F_c$  values greater than 10 rhes. The buret type of capillary instrument has an advantage over the single-volume viscometer type for the determination of the fluidity of complex cotton-cuprammonium dispersions with  $F_c$  values less than 10 rhes, since the velocity gradient adjustment is readily obtained with the buret consistometer in one discharge.

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# Antimony Trichloride Reaction of Vitamin D

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IN RECENT years, many investigators have reported on methods for the physicochemical estimation of vitamin D in fish liver oil. Many of these methods (1-4, 7, 8) depend upon the measurement of the yellow color developed when antimony trichloride or some modification (5, 6, 9) of this reagent is added to the vitamin D-containing fraction which is usually freed from vitamin A, sterols, and other color-producing substances by a chromatographic procedure.

Some years ago when these laboratories were actively investigating this problem, a reference calibration curve of the antimony trichloride yellow color was made up using crystalline calciferol (British Drug House) as a standard. It was found that variations in the conditions of concentration, time, light, and temperature all had a marked effect in the development of the yellow color at 500  $m\mu$ . Some of these effects have been overlooked by other investigators. These observations are published here in the hope that they may enable others to obtain closer agreement of their results.

## EXPERIMENTAL PROCEDURE

One milliliter of a chloroform solution of calciferol was measured into a colorimeter tube, and 10 ml. of a chloroform solution of antimony trichloride (saturated at 20° C.) were added rapidly from an automatic pipet. The intensity of the orange color was determined in an Evelyn photoelectric colorimeter, using a 500  $m\mu$  filter. (The filter was made up by E. E. Richardson of the Eastman Kodak Research Laboratories by adding some components to the 500  $m\mu$  filter supplied by the Rubicon Company to give a sharper band.) From the galvanometer reading function, approximately proportional to the optical density, was determined. Using this basic procedure, the conditions of concentration of calciferol, time, light, and temperature were varied.

## EFFECT OF VARYING CONDITIONS

**CONCENTRATION.** The intensity of the yellow color of the antimony trichloride-calciferol reaction product was not proportional to the amount of calciferol in the aliquot tested, except at very low concentration (5 micrograms or less per ml.). With larger amounts the color development was much less than would be expected from the intensity of color at lower concentrations (Figure 1, upper). These observations were confirmed by measurements made at 500  $m\mu$  on a recording spectrophotometer. Thus a calibration curve is recommended. If a conversion factor is used, it must be limited to a small range of optical density.

**TIME.** Calciferol-antimony trichloride colors must be measured after an exact interval of time. At 30° C. the color intensity reached a maximum after 4 minutes, then slowly and steadily faded (Figure 1, center).

**LIGHT.** The maximum color development was reached when the reaction was allowed to take place in the dark. When the color was developed in a shaded corner of the room on a bright day, the results were about 10% low, and when allowed to stand near the window, the results were about 15% low.

**TEMPERATURE.** Changes in temperature had a profound effect on the color development (Figure 1, lower). The intensity increased with temperature to a maximum at 42° C., beyond which it decreased. Between normal ranges of room temperature (19° to 33° C.) there was a difference of 40% in the color intensity of the same solution.

## DISCUSSION

The author is not attempting to set up strict conditions under which vitamin D determinations with antimony trichloride could be run. This is left to the individual investigator, but attention is called to the variables which must be controlled

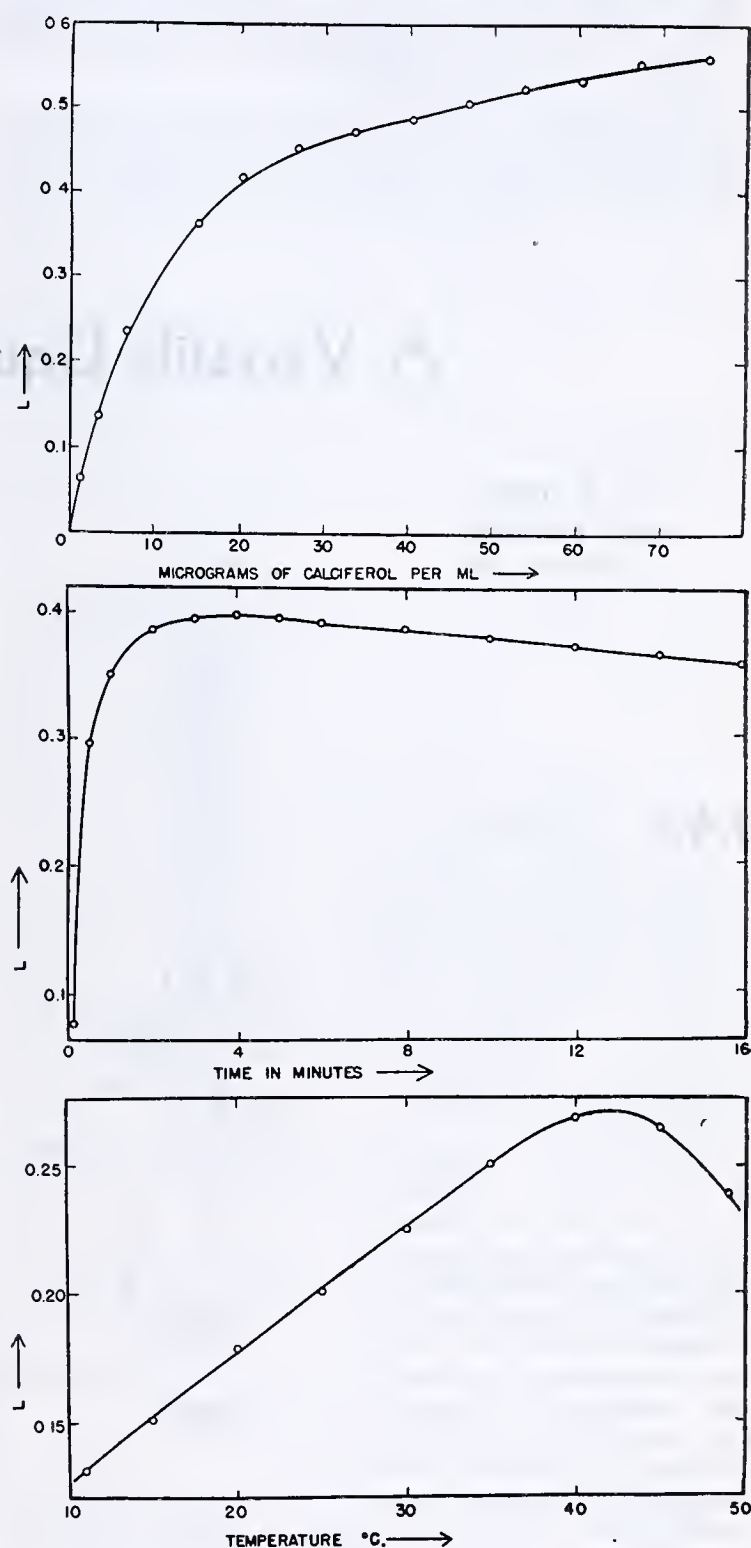


Figure 1. Intensity of Calciferol-Antimony Trichloride Color,  $L$ , at 500  $m\mu$

Upper. Plotted against concentration of calciferol  
Center. 0.0014% solution plotted against time  
Lower. 0.0008% solution plotted against temperature at which color was allowed to develop

closely if reproducible results are to be obtained. In this laboratory satisfactory checks were obtained ( $\pm 2\%$ ) on samples of calciferol by the following procedure:

One cubic centimeter of chloroform solution, calculated to contain about 0.07 to 0.25 mg. of calciferol, was measured into a colorimeter tube which had been allowed to come to constant temperature by inserting in a rack of steel tubes immersed in a



controlled temperature bath at 30° C. Ten cubic centimeters of antimony trichloride solution (saturated in chloroform at 20° C.) were then added. The reagent had also been previously brought to 30° C. by immersing the container in the constant-temperature bath. The steel tubes were covered to exclude light and the color was allowed to develop for exactly 4 minutes. The colorimeter tubes were removed and immediately read on an Evelyn photoelectric colorimeter, using a 500 m $\mu$  filter. The amount of vitamin D was calculated from a calibration curve prepared from crystalline calciferol.

In the estimation of vitamin D by the antimony trichloride procedure, the conditions of concentration, time, light, and temperature must be rigidly controlled to obtain reproducible results.

## A Versatile Liquid-Liquid Extractor

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**M**ANY liquid-liquid extractors have been described. A large number of these are patterned after the device described by Marshall (1) which, although labor-saving, is slow and therefore expensive to operate. In addition certain materials are heat-labile to the extent that they are destroyed in the boiling flask.

The apparatus herein described utilizes the well-known gas lift principle to overcome these objections and presents many additional possibilities for extraction procedures, catalytic reactions, adsorptions, etc. It is compact, simple to build, and very economical to operate. Low vacuum (0.5 inch) or a slight air or gas pressure serves equally well to operate the unit whether it be used as a small laboratory unit or for large-scale extractions. The extractant may be either the heavier or the lighter liquid.

The basic apparatus, which may be used as a batch extractor and which has been used to advantage in procedures normally carried out with separatory funnels, is shown in Figure 1. In operation, vacuum at 1 or pressure at 2 causes the lighter liquid in 3 to drop to 4, where a plug of air enters tube 5 and pushes the liquid in 5 into reservoir 6. Increased height of the liquid at this point causes a downflow through tube 7, where it bubbles out through a fritted-glass bubbler and rises to the liquid interface, 8, thence to point 4, momentarily sealing opening to tube 5. Gas entering through tube 3 again forces liquid into reservoir 6. A low vacuum or gas pressure causes a continuous, rapid bubbling action. When the heavier liquid is to be

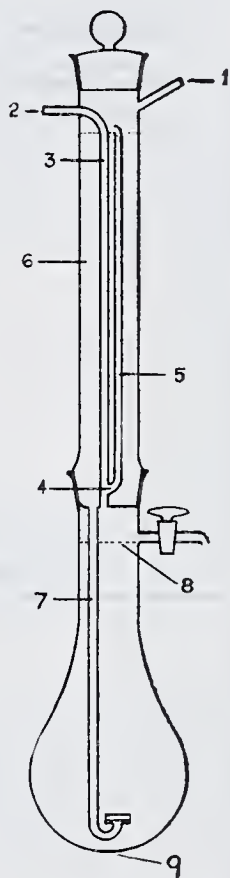


Figure 1

the extractant, an outlet should be provided at the bottom of the flask, 9.

Figure 2 illustrates the extractors used in conjunction with boiling flask and condenser for continuous operation. Extraction time is much reduced by this arrangement.

Figure 3 is a design of an apparatus utilizing this principle for extracting or percolating solid materials or for conducting absorptions or catalytic reactions. The cylinder, 1, may be packed or a thimble containing the material to be extracted may be inserted into the chamber. The unit is operated in the same manner as the liquid-liquid extractor.

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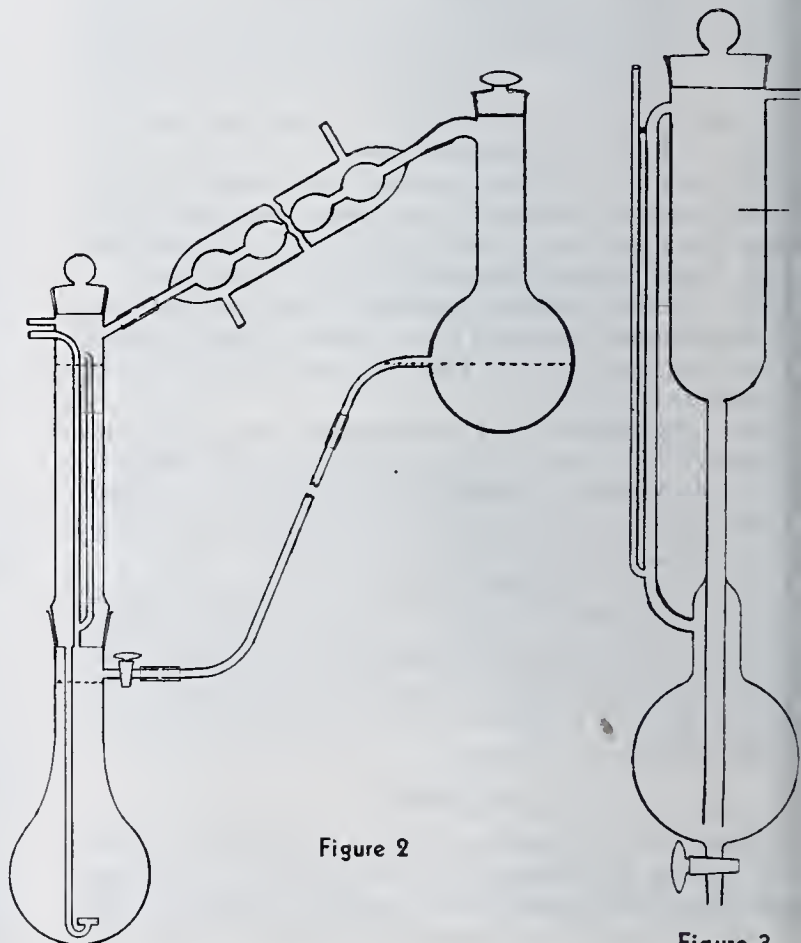


Figure 2

Figure 3



# Elimination of Nitrate Impurities from 30 Per Cent Hydrogen Peroxide

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THE phenol-disulfonic acid method for the determination of nitrates in plant tissue is being used in this laboratory. Any modifications have been reported.

Roller and McKaig (2) developed a modified method involving the use of 30% hydrogen peroxide as an oxidizing agent for organic matter. Because of the appreciable nitrate impurity in hydrogen peroxide, it was found necessary to purify this reagent, and distillation under reduced pressures was suggested by Roller and McKaig. [At the present time, 30% hydrogen peroxide low nitrates is sometimes difficult to obtain in large quantities on immediate request, and the commercial product—e.g., peroxol c.p. grade—obtainable in somewhat larger quantities, generally contains approximately 200 p.p.m. of nitrate.] This method of purification is time-consuming, and furthermore 30% hydrogen peroxide when distilled under reduced pressures (15 to 60 mm. of mercury) has seldom been found by the author to yield a distillate of more than 20% hydrogen peroxide. Consequently, attempts were made to purify the reagent by other means. Experiments indicated that this could be done by percolating the reagent through layers of activated carbon suitably separated as shown in Figure 1.

### APPARATUS AND PROCEDURE

A water-cooled adsorption column with dimensions as indicated is used. A sintered-glass filter plate sealed to the lower end of the column was found necessary to prevent small carbon particles from passing into the purified reagent. The column is loosely packed with 5- to 10-gram portions of activated carbon, Columbia brand activated carbon, type F, (size 20/48) alternating with glass beads, glass wool plugs, and perforated porcelain plates. The separatory funnel at the top of the column is filled with hydrogen peroxide, suction applied at the receiving flask, and the rate of percolation regulated by the stopcock on the separatory funnel to produce a desirable flow.

### DISCUSSION

It was necessary to design a column and to find a suitable adsorbent which could be used to remove nitrate from hydrogen peroxide without subsequent decomposition of this reagent. The effectiveness of various forms of active carbon was determined experimentally.

Very fine active carbon, such as powdered Nuchar or Norit A, was unsatisfactory in the author's column because of the slow rate of percolation of reagent, and the rapid rate of decomposition due to the particle size. A coarse product such as Nuchar 4/10-mesh was somewhat more suitable than a fine carbon, but did not compare favorably with the Columbia Carbon product. Commercial organic anion-exchange materials could not be used because of the rapidity with which they are oxidized and broken down by hydrogen peroxide. Under the author's working conditions, Columbia brand activated carbon appeared to be best adapted to requirements.

In order to obtain maximum efficiency, the carbon is pretreated as follows: A slow stream of sulfur dioxide is passed for several hours into a large Erlenmeyer flask containing 500 grams of carbon. The carbon is then oven-dried at 110° C. for 24 hours, and is ready for use. Active carbon not treated in this manner may be used, but the nitrate content of the purified peroxide is generally somewhat higher and varies from 15 to 30 p.p.m.

The percentage concentration of the final product appeared to be related to the rate of percolation of fluid (Table I). At a low rate of flow, appreciable decomposition occurred because of catalytic activity of the carbon. As the rate was increased, the percentage composition tended to approach that of the original reagent. Consequently, it may be necessary to determine by trial and error at what rate the reagent should be percolated through the column in order to yield best results. The author has found that for his apparatus a rate of percolation of approximately 200 cc. per minute is most effective.

A water-cooled system was considered necessary since heat is liberated at the carbon-fluid interface. The rate of decomposition is increased appreciably with increase in temperature, and an explosive rate may be approached if an air-cooled unit is used (1).

The purification of the reagent is obviously dependent upon the nature of the adsorbing agent; however, the manner in which the column is packed and the thickness of the carbon layers, as well as the distance between them, are important in maintaining proper temperatures, a rapid rate of percolation, and, subsequently, a concentrated purified product.

### RESULTS

By passing successive portions of hydrogen peroxide through the column, it was found that approximately 35 grams of active carbon were sufficient to purify over 500 cc. of the reagent. The

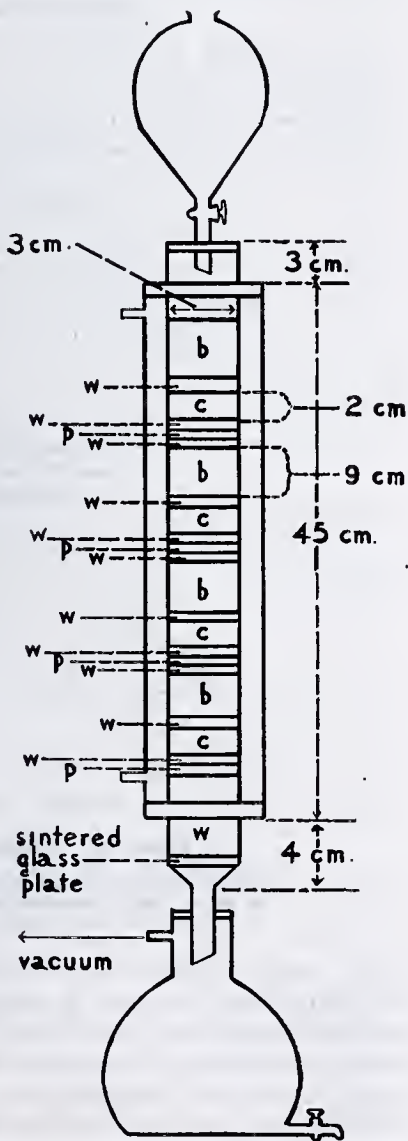


Figure 1. Adsorption Column  
b. Glass beads  
c. Carbon  
p. Perforated porcelain disk  
w. Glass wool

Table I. Nitrate in Hydrogen Peroxide

(Column packed as indicated in Figure 1. Original NO<sub>3</sub> content, approximately 200 p.p.m.)

Rate of Percolation of H <sub>2</sub> O <sub>2</sub> Cc./min.	H <sub>2</sub> O <sub>2</sub> in Original Product %	H <sub>2</sub> O <sub>2</sub> in Purified Product %	NO <sub>3</sub> in Purified Product P.p.m.	Temp. of Purified Reagent ° C.
Thickness of Carbon Layers, 2 Cm. Distance between Layers, 9 Cm. No. of Carbon Layers, 4				
200	31	28	6	Approximately 23
100	31	24	5	Approximately 23
60	31	21	5	Approximately 23
Thickness of Carbon Layers, 4 Cm. Distance between Layers, 5 Cm. No. of Carbon Layers, 4				
200	31	23	4	Approximately 23
100	30	19	4	Approximately 23
60	30	16	5	Approximately 23
Column Packed with Glass Beads Thoroughly Mixed with 35 Grams of Active Carbon				
100 <sup>a</sup>	29	5	6	60
50	31	2	6	60-70

<sup>a</sup> A rate of percolation of 200 cc. per minute was not attained. Decomposition of H<sub>2</sub>O<sub>2</sub> interfered with rapid percolation of fluid.



purified product contained less than 10 p.p.m. of nitrate and its average percentage concentration was approximately 27 to 29% hydrogen peroxide. [The nitrate content of both untreated and purified hydrogen peroxide was determined according to Scott (3). An aliquot of hydrogen peroxide containing 0.1 mg. or less of nitrate was used.]

No investigations were made as to a method of "recharging" the carbon by replacement of adsorbed nitrate with other anions, so that carbon might subsequently be utilized again. Once the column has become saturated with nitrate, its contents are replaced with fresh material.

The nitrate content of the purified reagent offers no difficulty in the determination of nitrates (the small amount present can be

accounted for in the blank determination), the peroxide concentration remains sufficiently high to produce the desired result and the time necessary for the purification of the peroxide has been reduced appreciably.

#### ACKNOWLEDGMENT

The author desires to express appreciation to T. C. Broyer for suggestions on this paper.

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## Operating Procedure for Determining the Heat of Combustion of Gasoline

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The paper describes details of operating procedure for determining the heat of combustion of gasoline. The degree of precision and the economy of conducting the tests compare favorably with those possible for petroleum products of low volatility.

MANY of the specifications for aviation gasoline now in effect in the United States include a minimum limit for lower or net heat of combustion. The prescribed procedure is to determine the gross calorific value in an approved oxygen-bomb calorimeter and obtain the net value by making a suitable correction for the latent heat of vaporization of water formed during combustion of the fuel.

Practically all published directions for determining the gross calorific value of aviation gasoline are exemplified by Method 250.2 of the Federal Specification for Lubricants and Liquid Fuels (2). This prescribes the use of the standard A.S.T.M. method of test for thermal value of fuel oil (1) modified as follows for volatile fuel:

Fill a dry, weighed, gelatin capsule of suitable size with dry cotton fiber, weigh the capsule again, and record the weight of gelatin and cotton. Fill the capsule by immersing it in the fuel and closing it under the surface. Dry the outside of the capsule, weigh the capsule immediately, and record the weight of the fuel. Wrap the ignition wire around the capsule three or four times and place the capsule immediately in the bomb. Fill the bomb with oxygen at 30 atmospheres pressure, and proceed with the test as outlined in Method 250.1.

Repeat the test if traces of sooty deposit or odor of unburned fuel are noticed when the bomb is opened after combustion.

Corrections. Make all corrections outlined in Method 250.1 and in addition correct for the heat of combustion of the gelatin and the cotton by subtracting from the total heat developed.

These directions, obviously, leave a great deal to the imagination of the operator. The authors' laboratory has had occasion to acquire a large amount of experience with this particular determination, and the present paper discusses the details which are missing in the published directions quoted above, and gives specific instructions followed by their operators.

#### CONDITION AND SIZE OF GELATIN CAPSULES

The use of a dry gelatin capsule of suitable size is prescribed. As purchased, capsules contain a large and indeterminate amount of water. One batch dried in a desiccator over calcium chloride showed a loss of weight of about 10% for the first day, and an additional 4.5% in the 20 days following. Complete drying could be effected by heating in an oven at 221° F., but the capsules then became brittle and unusable. It was found that capsules "as received" did not change appreciably during the normal time of the weighing operation, regardless of whether the

humidity was high or low, and it appeared that the important feature was to ensure uniform water content in a batch, and avoid extraneous surface moisture.

Capsules having a suitable capacity of about 0.1 ml. designated as "No. 00 size" by at least one commercial supplier

#### FILLING OF CAPSULES

The published directions call for filling a weighed capsule containing a determined quantity of dry cotton, by immersing it in the fuel and closing it under the surface of the liquid. The cotton serves to minimize splashing when the capsule is ignited

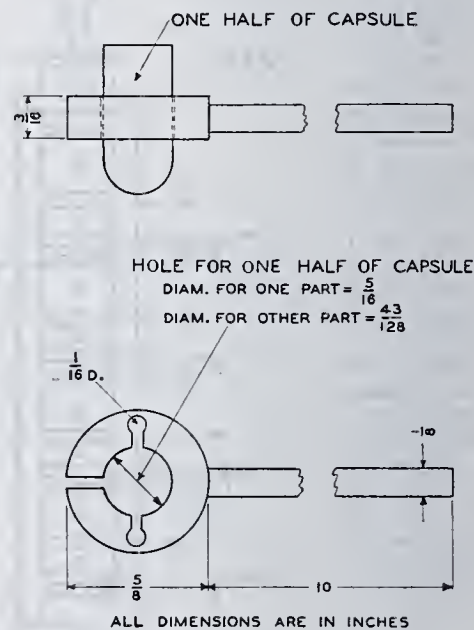


Figure 1. Capsule Holder

Consists of two parts, one shown with half-capsule in place. Inside diameters, as shown, are suitable for capsules used in authors' laboratory.

in the bomb. Ignited asbestos serves the same purpose and has the obvious advantage of contributing nothing to the heat generated during the combustion period. However, by observing certain simple precautions it is possible to dispense with cotton or asbestos. Splashing does not occur if the capsules are completely filled with liquid and free from even the smallest bubble of air or other permanent gas.

The prescribed operation of filling by pushing together the two halves of the capsule below the surface of the liquid fuel can, if necessary, be performed with the fingers. The use of a spec-



holder, such as is shown in Figure 1, has obvious advantages, particularly as regards the size of the container and the quantity of fuel required. It is not considered good practice to fill more than one capsule from the portion of gasoline which has been burned into the dish.

#### EVAPORATION LOSSES

The fact that capsules are not perfectly tight is recognized in the published directions, by the provision for weighing "immediately" after filling, and placing the capsule in the bomb "immediately" after adjusting the ignition wire. This is to minimize evaporation losses which change the composition of the fuel and introduce an uncertainty as to the weight of the charge actually burned in the bomb. The error due to change in composition of the gasoline seems to be unavoidable but is of negligible magnitude when dealing with the present types of ignition fuel and is perhaps 0.02 or 0.03% for each full per cent evaporation loss. The error due to loss of material is in direct proportion to the amount of gasoline that is vaporized.

A simple practical scheme, devised for determining the evaporation loss between the time of completing the final weighing of the filled capsule and the time of closing the bomb, involves determining the rate of evaporation loss (rejecting the capsule if this is excessive) and measuring the time interval between the completion of the weighing and the closing of the bomb. When the initial evaporation loss is not in excess of 0.0010 gram per minute, the rate remains practically constant for at least 2 additional minutes. An experienced operator can usually transfer a capsule from the pan of the balance to the bomb in one minute or less.

#### DETAILED WORKING DIRECTIONS

**PREPARATION OF SUPPLY OF CAPSULES.** Place approximately 100 gelatin capsules in a bottle with a tight screw top. Allow to stand for at least 30 days to ensure equalization of the moisture content of all individual capsules. In case of emergency, this "soaking" period may be reduced to one week by using an oversize bottle and shaking its contents vigorously at least twice a day.

At the time the soaking period is started, prepare test portions for determination of the calorific value of the gelatin. Each portion consists of about ten capsules, cut into small pieces and placed inside a capsule. Put these test portions, with the halves of the containing capsules separated, in a small shallow dish or a perforated gill can, which is placed in the bottle with the main supply. At the end of the soaking period, remove the can, assemble the capsules, and place them in a small bottle which is tightly closed. Remove the gelatin-filled capsules from this bottle one at a time for determination of their calorific value. Make at least three determinations, and if the average deviation from the mean of these results exceeds 45 B.t.u. per pound, make additional determinations until the average deviation is less than this amount.

Transfer at least 100 capsules from the large bottle to a set of small screw-top bottles of similar type, putting 10 to 15 capsules (approximately 2 days' supply) in each bottle. Avoid making the transfer under conditions which might cause the capsules to pick up surface moisture. The relative humidity should not be too high and the capsules should not be far enough from room temperature to bring about condensation.

**FILLING OF CAPSULES.** Withdraw a capsule from the current working supply, restoppering the bottle immediately. Weigh with an accuracy of 0.1 mg. and record as  $W_1$ . Pour some of the sample to be tested into a small beaker or crucible. Place each half of the capsule in a suitable brass holder such as is shown in Figure 1. Immerse the halves of the capsule in the gasoline, with the open ends obliquely upward, and agitate very gently for 20 to 30 seconds, to get rid of bubbles of occluded air or other permanent gas which would interfere with complete filling. Then draw the two halves together, still keeping them immersed. Withdraw from the liquid, wipe dry with a clean cloth, and place in the pan of the balance.

Weigh to 0.1 mg. and record as  $W_2$ . When balance is attained, start a stop watch and obtain another weight exactly 60 seconds later. Record as  $W_3$ . Insert the filled capsule in a coil of ignition wire, already attached to the terminals of the bomb and push it down into the cup or crucible. The coil is

made by wrapping three or four turns of ignition wire around a rod of the same diameter as the capsules. Care should be taken not to warm the capsule appreciably while transferring from the balance to the coil of ignition wire. If the operator uses bare fingers, they must be dry and his touch must be light. Other precautions, such as the use of special tongs, are obviously permissible. Stop the watch when the lid of the bomb is in place. Do not include the operations of screwing on the lock ring, etc., in the time period thus measured.

Compute the weight of gasoline burned,  $W_b$ , from the following formula:

$$W_b = (W_2 - W_1) - (W_2 - W_3) \frac{t}{60}$$

$t$  is the time in seconds elapsed between completion of the first weighing of the filled capsule and the closing of the bomb, specifically, it is the stop-watch reading.  $(W_2 - W_1)$  is the weight of gasoline originally in the capsule and  $(W_2 - W_3)$  is the amount lost by evaporation during the 60-second period the filled capsule was kept on the balance pan. A normal figure for this loss is from 0.0001 to 0.0010 gram. If it is as much as 0.0015 gram, or if any bubble of air or vapor is observed in the capsule before the bomb is closed, the capsule is rejected.

Obtaining a weight at some predetermined instant is easy with various types of automatic-reading balances now available. If the simple conventional type of balance is used it is recommended that its sensitivity be determined and that  $(W_2 - W_3)$  be measured in terms of change in the swing rather than by trying to shift the rider. It is, however, possible to measure the time for, say, 0.0010-gram loss rather than the loss during 60 seconds. If this is done, the formula above should be suitably modified.

#### OTHER METHODS FOR BOMB-CALORIMETER DETERMINATIONS WITH VOLATILE FUELS

A somewhat different procedure, using gelatin capsules, has been described by Jones and Starr (4). It has not been tried in the authors' laboratory but they believe it is slightly less convenient and no more accurate than that described above.

The method of Richter and Jaeschke (7) involves weighing the charge of gasoline in a special platinum crucible covered with a thin skin of collodion. It has not been tried, but the accuracy claimed is no better than that normally attained with the procedure described above.

The most precise method known to the authors is that originally described by Richards and Barry (6), and later improved by Jessup (3) and Prosen and Rossini (5). The charge is weighed in a hermetically sealed special glass bulb with flattened sides. This bulb, if properly made and completely filled, opens in the bomb without splashing, when exposed to the heat generated by the combustion of the iron fuse wire. The procedure is more time-consuming than that described above and the technique of fabricating, filling, and sealing the bulbs requires a higher degree of manipulative skill than is necessary when using gelatin capsules.

The authors take this opportunity to suggest that the ideal solution of the problem is to weigh the charge in a special closed container which opens mechanically in the bomb when the ignition wire is burned. With adequate ingenuity and experimentation a practical device of this type might be developed.

Table I. Benzene Blank Determination for a Typical Period of 29 Consecutive Working Days

(Values in British thermal units per pound)

18,032	18,020	18,005	18,071
18,030	18,031	18,057	18,057
18,011	18,069	18,043	18,039
18,032	18,065	18,048	18,045
18,048	18,034	18,067	18,030
18,041	18,057	18,045	18,033
18,042	18,005	18,060	18,058
18,010			

Mean, 18,041

Av. deviation from mean, 15.5 B.t.u., or 0.086%

Max. deviation from mean, 36 B.t.u., or 0.200%



## PRECISION ATTAINABLE

It is customary in the authors' laboratory to make a blank determination once a day on a sample of "chemically pure" benzene. Table I shows the results obtained in a period, selected at random, of 29 consecutive working days.

The exact calorific value of the benzene used is not known, hence these "blank" determinations are an index of the reproducibility rather than the absolute accuracy of the determinations. The basic standard is benzoic acid, obtained from and certified by the National Bureau of Standards.

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# Chromatographic Determination of Carotene in Alfalfa

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A chromatographic method is presented, with supporting experimental data, which avoids the oxidative losses of carotene generally encountered in freshly harvested plant tissues, as well as errors due to incomplete extraction and incomplete separation of carotenes from other pigments present. The method includes an enzyme in-

activation and sample storage procedure, making possible the collection and preparation of large numbers of samples on fixed dates. The chromatographic technique has been modified for the purpose at hand, by converting the adsorption column to an adsorption filter which avoids losses of adsorbed carotene.

IN THE assay of plant tissues for carotene three important factors are capable of causing large errors in determined values. (1) The carotenes are unstable, undergoing oxidation under ordinary conditions and isomerization or other changes (1) under certain conditions. This necessitates the use of proper precautionary measures throughout the analysis. (2) Difficulty is encountered in many instances in extracting all the carotene. Each different plant tissue must be handled as a separate extraction problem and checks must be carried out to prove that extraction has been complete. (3) Separation of carotenes from other substances in the extract prior to measurement is difficult. The carotenes occur in intimate association with other carotenoids whose close chemical and physical relationship to the carotenes makes their elimination a serious problem. Several investigators (4, 7, 11, 12) have concluded that in methods based on phasic separations, noncarotene pigments are measured as carotene. The chromatographic technique appears to be the only one extant which is capable of completely removing these interfering substances.

A method for routine use has been evolved which overcomes for practical purposes all the difficulties referred to and requires only very simple equipment. Using this method one operator can analyze eighteen prepared alfalfa samples per 8-hour day. While modifications have been applied to a variety of plant tissues, the discussion from this point on deals principally with the determination as applied to freshly cut alfalfa.

## PROCEDURE

**FIELD SAMPLING.** From each field plot to be sampled a representative sample of about 1000 grams is collected by cutting off within an inch of the ground small handfuls of alfalfa at a number of random positions throughout the plot. Immediately after collection the sample is wrapped in paper with enough cracked dry ice (as shown by experience) to freeze it quickly and maintain a frozen condition until subsampling and enzyme destruction can be carried out. The resulting bundles of alfalfa rolled in paper are tagged and placed in an insulated double plywood box for transportation to the laboratory.

**SUBSAMPLING AND ENZYME DESTRUCTION.** Each field sample is taken from the box the same day as cut and while still frozen is unwrapped and run twice through a Russwin food chopper (not a meat grinder) with any remaining dry ice, then mixed thoroughly by hand to render it as homogeneous as possible. At this

point all desired subsamples, about 5 grams each, are weighed immediately and directly into 125-ml. Erlenmeyer flasks containing about 25 to 30 ml. of the slightly alkaline ethanol preparation described below. The flasks are shaken briefly and placed on a hot plate, where they are allowed to simmer under a reflux condenser for 10 minutes. After partial cooling the flasks are completely filled with Skellysolve B, stoppered tightly with rubber stoppers, and placed in a refrigerator at  $-1^{\circ}\text{C}$ . until taken for analysis.

**EXTRACTION.** At any convenient time, as long as 30 days thereafter, the entire contents of each flask are transferred quantitatively to the container of a Waring Blendor with the aid of a stream of Skellysolve B from a wash bottle. A convenient type of wash bottle for this purpose has been described (2). The Blendor is run for about 2 minutes, after which the contents are filtered directly into a 500-ml. separatory funnel. A convenient filter for this purpose is a funnel large enough to accommodate the entire contents of the Waring Blendor container, fitted with a piece of sheeting or other closely woven white cotton cloth. The solids retained by the filter are washed with Skellysolve and squeezed out repeatedly therein.

**PREPARATION OF SOLUTION FOR CHROMATOGRAPHING.** After the lower layer is discarded, the resulting solution must be freed of most of the chlorophyll present and of other polar solutes since these would interfere with the subsequent chromatographic separation if allowed to remain. This is accomplished by washing the solution repeatedly according to the following directions:

1. Add 25 to 30 ml. of 10% (weight per volume) solution of potassium hydroxide in 80% ethanol and shake the separatory funnel vigorously for 4 or 5 seconds. Discard the lower layer.
2. Repeat (1) once.
3. Add about 100 ml. of water and shake gently for a few seconds. Discard the lower layer.
4. Add about 100 ml. of approximately 4% sulfuric acid solution and shake gently for a few seconds. Discard the lower layer.
5. Add about 200 ml. of water and invert the funnel three or four times quickly but without undue force. Discard the lower layer.
6. Repeat (5) once.
7. Finally, immediately before chromatographing, draw off and discard the last water which settles.

These operations may be performed rapidly. Only one separatory funnel is required for each sample, since it is always the lower layer which is discarded. The carotene solution remains in the same funnel and no time-consuming transfers are necessary. All the separations of layers referred to take place rapidly unless preceded by too vigorous shaking, which is unlikely to occur except in the two final rinsings with water. In practice six carotene determinations are carried out simultaneously. With a battery of six separatory funnels, no waiting for separation of layers is necessary. All the discarded layers have been analyzed repeatedly and have never been found to contain measurable amounts of carotene.

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**CHROMATOGRAPHIC SEPARATION OF CAROTENE.** After the last bit of water which separates is discarded, the contents of the separatory funnel are passed through a drying tube charged with anhydrous sodium sulfate placed above an adsorption tube packed with an adsorptive mixture (1 part of magnesia to 8 parts of soda ash by weight) into a 10-ml. graduated cylinder connected for removal of air by suction (see Figure 1). The percolation cannot be speedily accomplished by gravity alone. The drying and adsorption tubes should be wetted previously with Skellysolve B. As soon as all the solution from the separatory funnel has passed into the drying tube, the funnel is rinsed with 25 to 30 ml. of Skellysolve B which is also passed into the percolation system. The system is now washed further with Skellysolve containing 1% ethanol by volume until the upper pigment bands begin to move down. This guarantees that no carotene, adsorbed or unadsorbed, remains in the column.

The adsorption tubes are prepared by packing the adsorbent mixture into filter tubes of about 20- to 25-mm. inside diameter, plugged at the constriction by a pad of cotton or glass wool. A depth of 1 cm. in a 20-mm. tube is adequate for carotene determinations in 5-gram fresh alfalfa samples. The tubes are packed by pressing small portions of the adsorbent firmly into place in the tube supported vertically. This packing is done with a plunger consisting of a long stout rod and a properly fitting rubber stopper. When the tube is held in a horizontal position, the surface layer retains its position it has been packed tightly enough.

**PHOTOMETRIC MEASUREMENT OF CAROTENE.** After percolation is complete the volume of the percolate is noted and its carotene content is measured in a photometer previously standardized against pure  $\beta$ -carotene or a suitable secondary standard solution. In this laboratory an Aminco type F photoelectric photometer was used routinely with the No. 42 filter in place. From the data thus obtained and the weight of the sample, the carotene content of the material analyzed is calculated.

## EXPERIMENTAL

**SAMPLING AND EXTRACTION.** Since the samples for experimental work at this station had to be collected in large numbers on seven days during the growing season, some method of storing them without loss of carotene prior to analysis was necessary. Preliminary work showed (1) that cold storage of samples in an atmosphere low in oxygen cannot be relied on to preserve carotene in alfalfa samples; and (2) that chopping alfalfa for representative sampling initiates very rapid destruction of carotene. Twenty-one samples which had been run through a food chopper followed in 16 hours an average loss of 37.2% of their carotene.

Rapid losses of this kind probably take place only in the presence of oxidases which occur in the plant. If so, alfalfa samples for carotene assay must be treated immediately after they are weighed out by some solvent or reagent capable of destroying the enzymes. Since Zimmerman *et al.* (13) recommended boiling in acetone as an enzyme-destroying agent and solvent, it was tried on alfalfa samples. Other agents tried were ethanol, and hot and cold ethanolic potassium hydroxide (5, 9). In all cases the volume used was 25 ml. on a sample weighing  $5 \pm 0.5$  grams. The results are shown in Figure 2.

The destruction of carotene is evident in samples heated with either diacetone or alcohol. Noteworthy is the fact that a strongly alkaline reagent, 10% ethanolic potassium hydroxide, gave higher values than either of the other solvents. This fact and the report of Beadle and Zscheile (1) that acids cause destruction of carotene, as well as the further fact that the ethanol

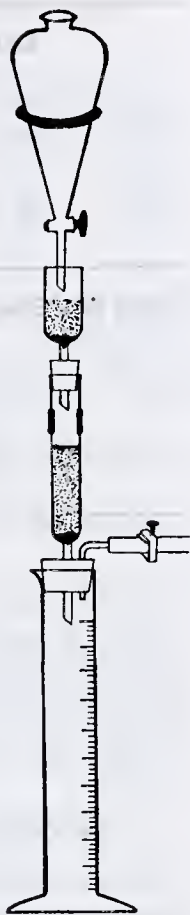


Figure 1. Adsorption Apparatus

used in the preceding experiment carried slight acidity, led to a trial of ethanol containing in solution a small excess of potassium hydroxide. Such a solution was prepared by adding to commercial ethanol enough powdered potassium hydroxide to neutralize its acidity, then 0.5 gram per liter excess.

Several 5-gram subsamples taken under the same conditions from a single well-mixed sample of ground alfalfa were weighed into 125-ml. Erlenmeyer flasks containing 25 ml. of the alkaline ethanol described above. The flasks were placed on a hot plate and left there for the contents to boil for 10 minutes. Since it was also desired to determine the effect of excluding air, the subsamples thus treated were divided into two lots. The flasks in one lot were completely filled with Skellysolve B in order to exclude air and were tightly stoppered; those in the other lot were stoppered tightly with the air unreplaced. All were stored in a refrigerator at  $-1^{\circ}\text{C}$ . to await analysis. At convenient intervals thereafter sets of four subsamples comprised of one pair from each of the two lots were analyzed. As controls, two other subsamples were taken and analyzed immediately without treatment. Figure 3 presents an average of results obtained in several repetitions of this experiment, in which carotene values have been reduced to percentages of the original content.

No great loss of carotene occurred, either during or after treatment with alkaline ethanol, if the samples were protected from air. If they were not so protected, a slow loss of carotene took place, presumably through spontaneous oxidation, since oxidative losses of carotene in the presence of enzymes appear to be much more rapid than observed here. It may be concluded that the treatment described prevents enzymatic and spontaneous oxidation of carotene in alfalfa samples without serious loss of carotene during treatment.

The foregoing comprises a part of the extraction procedure, since after samples are so treated and stored for a few days under the stated conditions they appear to have undergone a softening or disintegration and to have lost their green color to the supernatant solvents. Subsequent maceration of samples in this condition is accomplished with

great ease in about one minute by means of a Waring Blendor, which is also recommended by other workers (3, 8).

**CHROMATOGRAPHY.** In the search for a suitable adsorbent for chromatographic separation of carotene from other extracted pigments, it was found that mixtures of soda ash (6, Merck technical) with magnesia (10, Micron brand) showed desirable properties; and that the adsorptive strength can be varied through a considerable useful range of mixture proportions. Inasmuch as the carotenes are subject to destruction while adsorbed (10) and are the least strongly adsorbed of all the pigments present, it was considered desirable so to adjust the composition of

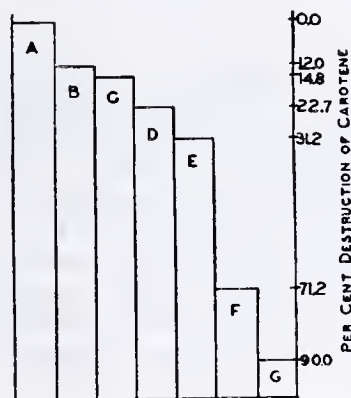


Figure 2. Effect of Treatments on Carotene in Alfalfa

- A. No treatment, analyzed at once (taken as 100%)
- B. Refluxed 20 minutes with 10% alcoholic KOH
- C. Digested 20 hours at room temperature with 10% alcoholic KOH
- D. Heated to boiling, boiled 10 minutes with alcohol
- E. Heated to boiling, boiled dry with alcohol
- F. Heated to boiling, boiled 5 minutes with diacetone
- G. Heated to boiling, boiled dry with diacetone

the adsorbent mixture that the carotenes are not adsorbed, but pass through the column while the other pigments are retained. The mixture which just fails to retain carotene was found to be one containing 8 parts of soda ash to 1 part of magnesia by weight. In chromatographing with this adsorbent the mixed carotene band is seen only fleetingly if at all. Thus the adsorption column



becomes in actuality an adsorption filter, and the possibility of loss of adsorbed carotene is eliminated.

The adsorbent mixture is easily reclaimed for further use by placing it in 9-cm. porcelain crucibles in a cold muffle furnace and bringing it gradually to a "low red heat". After 1 or 2 hours at this temperature the furnace is turned off and the contents are allowed to cool in place. This slowly burns out the adsorbed organic substances without destroying the adsorbent, which can be reclaimed in this way at least six times without noteworthy change in activity.

## RESULTS AND DISCUSSION

**ACCURACY OF THE METHOD.** As a measure of accuracy of the method standard deviations were calculated from duplicate values which were available for each of the three cuttings of alfalfa (Table I). The standard deviations are roughly proportional to the mean values found for the three cuttings. This indicates that the inherent precision of the method is at least as great as the accuracy with which the samples were taken.

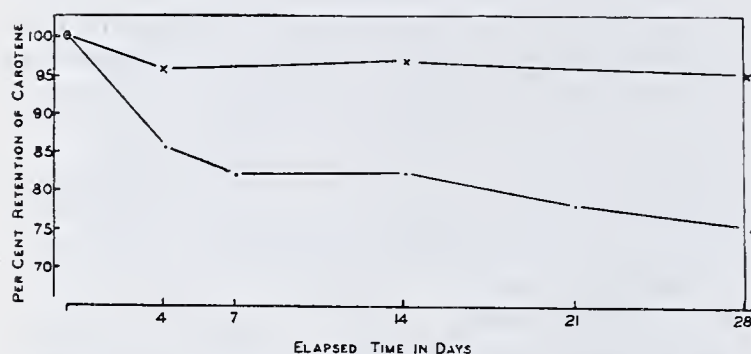


Figure 3. Effect of Storage Conditions on Carotene in Alfalfa

- No treatment, analyzed at once (taken as 100%)
- Heated 10 minutes with boiling "alkaline alcohol", then stored at -1° C. in stoppered flask
- X Heated 10 minutes with boiling "alkaline alcohol", then stored at -1° C. in stoppered flask completely filled with Skellysolve B

**RECOVERY OF ADDED CAROTENE.** A number of recovery tests were run by assaying fresh alfalfa samples along with duplicates to which were added known quantities of pure  $\beta$ -carotene (Smaco) in the form of a concentrated solution in Skellysolve B. The amount of carotene added was determined by measuring photometrically a diluted aliquot of the same concentrate. The results are shown in Table II, where all values for carotene found are averages of two or three separately determined values. A small loss of carotene occurs during analysis by this method. This is not surprising in view of the labile nature of carotene, particularly its great susceptibility to oxidation.

Table I. Standard Deviations of Duplicate Carotene Values in Alfalfa

Cutting	No. of Pairs of Values, $n$	Sum of Squares of Deviations, $\Sigma x^2$	Mean of All Values Obtained, $\gamma/g.$	Standard Deviation of a Single Value, $\sigma$
1st	17	65.15	34.5	2.02
2nd	27	146.94	48.7	2.375
3rd	13	136.40	68.4	3.37

Since in the foregoing recovery tests all samples, with and without added carotene, were subjected to the enzyme-inactivating heat treatment with alkaline ethanol, compensating losses of carotene may have occurred during the heating. Table III shows a comparison of duplicate average values found on subsamples of the same lots of fresh alfalfa analyzed with and without this heat treatment. Unheated subsamples were extracted immediately

Table II. Recovery of Added Carotene

Carotene Sample + increment $\gamma/g.$	Found Sample - increment $\gamma/g.$	Difference $\gamma/g.$	Wt. of Sample Grams	Carotene Recovered $\gamma$	Carotene Added $\gamma$	Recovery %
74.37	66.02	8.35	5.30	44.25	47.0	94.1
98.34	43.03	55.31	5.00	276.55	283.5	97.5
92.3	48.23	44.07	5.09	224.3	220.3	101.8

Table III. Effect of Enzyme-Inactivation Treatment on Carotene

Sample No.	Heated $\gamma/g.$	Not Heated $\gamma/g.$	Loss on Heating $\gamma/g.$	Destruction %
1	62.9	64.0	1.1	1.7
2	54.1	56.6	2.5	4.4
3	66.0	67.35	1.35	2.0
4	61.4	63.5	2.1	3.3

to avoid enzyme action. These results indicate a small loss of carotene during treatment, which is not serious for practical purposes and which could be eliminated only by elaborate precautions. According to the results presented, the method described here measures carotene in fresh alfalfa with an accuracy of more than 90% and does not give erroneously high values.

## APPLICATIONS TO PRODUCTS OTHER THAN ALFALFA

Since the method was first applied to fresh alfalfa and most of the experimental data were collected using samples of this material, this report has been written accordingly. However, modifications have been applied to a large number of other plant varieties, usually with greater reproducibility of results than reported here. This follows from the apparent greater oxidase activity of alfalfa juice, its more complex pigment system, and its physical form which makes accurate sampling difficult. The modifications referred to are usually in the size of the sample or method of extraction, with concomitant adjustments of adsorbent volume used or of adsorbent mixture proportions. Any adjustment of mixture proportions is governed by the nature of the separation involved—for instance, in the determination of carotene in tomatoes the carotenes must be separated from lycopene. The properties of lycopene, another hydrocarbon, resemble those of the carotenes more closely than do those of the carotenols, etc., found in alfalfa. This separation is therefore usually regarded as a more difficult one; however, it is accomplished easily by use of an adsorbent mixture containing 1 part of magnesia to 6 parts of soda ash by weight. The use of a mixture of this strength permits actual observation of the carotene, which is adsorbed temporarily but can be readily removed by further washing with Skellysolve B.

## ACKNOWLEDGMENTS

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# Determination of Manganese after Oxidation to Tri-Dihydrogen Pyrophosphatomanganate

## Use of Pyridine to Separate Iron, Chromium, Vanadium, and Cerium from Manganese

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Vanadyl, vanadate, ceric, cerous, and chromic ions are quantitatively coprecipitated with an excess of ferric iron when hydrous ferric oxide is precipitated by pyridine. Chromate divides between the precipitate and filtrate. Manganous and ferrous ions remain quantitatively in the filtrate. Use of pyridine is advantageous in separating the interfering metals, vanadium, chromium, cerium, and a large excess of iron from manganese prior to the polarographic determination of manganese as tri-dihydrogen pyrophosphatomanganate.

IN APPLYING the authors' (1) polarographic procedure for the determination of manganese, after its oxidation to tri-dihydrogen pyrophosphatomanganate, to the analysis of ores and steels it was found that large amounts of iron and even small amounts of chromium, vanadium, and cerium interfered with the determination. These elements must be separated from manganese before the polarographic procedure can be applied. It will be shown in a subsequent paper that as little as 1 or 2 mg. of manganese can be determined in the presence of 0.2 gram of iron without a separation; if more iron is present, a separation is necessary. The interference of chromate can be eliminated by reduction with arsenious acid. Otherwise the pyridine separation was found to be the most effective and simple method for eliminating the interference. In this procedure chromium, vanadium, and cerium are removed from solution by coprecipitation with hydrous ferric oxide.

Pyridine, a very weak base having an ionization constant of  $4 \times 10^{-9}$ , has been recommended for the quantitative precipitation of certain trivalent metals as the hydrous oxide without appreciable coprecipitation of cobalt, nickel, manganese, and copper (2, 3). A mixture of approximately equal concentrations of the pyridinium salt and pyridine is well buffered and is slightly acidic, having a pH of approximately 5.2 at 25° C. The solid hydrous oxide thus acquires a positive charge due to its primary sorption of hydrogen ions. Consequently anions such as the sulfate ion, and not cations such as cobalt and nickel, are adsorbed as counter ions and are carried down with the precipitate. A second property of pyridine undoubtedly contributes to the excellent separations of the hydrous oxides from cobalt, copper, nickel, and manganese. The pyridine molecule, owing to the lone pair of electrons on the nitrogen atom, is able to occupy a place in the coordination sphere in complex-forming metal ions. The great many complexes of the above metals with pyridine are known. The authors found that the formation of a complex of chromic chromium with pyridine actually is a factor in preventing

the precipitation of chromic ion in the absence of ferric iron or aluminum.

In order that the pH shall not exceed 5.2 when 5 ml. of pyridine are added to approximately 25 ml. of aqueous solution, it is necessary to have 31 milliequivalents of strong acid or its equivalent of metallic ion such as ferric iron present. Each millimole of ferric iron liberates 3 milliequivalents of acid upon precipitating as the hydrous oxide. If 0.6 gram of ferric iron were present, no excess of mineral acid would be necessary. For each 0.1 gram of ferric iron present, the amount of strong mineral acid present should be decreased by 5 milliequivalents. However, 5 ml. of pyridine will precipitate quantitatively as much as 1.0 gram of ferric iron from 100 ml. of solution containing no appreciable excess of acid.

### APPLICATIONS

The use of pyridine to separate quantitatively trivalent chromium, iron, or aluminum from manganese, cobalt, and nickel; or uranium from calcium, barium, and strontium, has been recommended by Ostroumov (3). According to him, a sum of 0.1264 gram of the oxides of trivalent iron, chromium, and aluminum may be quantitatively separated from a solution containing about 0.02 gram of divalent manganese, cobalt, or nickel with less than 0.2% coprecipitation of these ions with the mixed hydrous oxides. Zinc, if present, divides between the precipitate and solution. Lingane and Kerlinger (2) more recently used a similar procedure to separate ferric iron and chromic chromium from copper, cobalt, and nickel before determining the last three elements polarographically.

One interesting difference was observed in the results of Ostroumov and those of Lingane and Kerlinger. According to Lingane and Kerlinger, trivalent chromium is separated only through coprecipitation with the hydrous ferric oxide, while Ostroumov found that trivalent chromium can be quantitatively precipitated in the absence of iron.

The following factors undoubtedly account for this difference. In Ostroumov's experiments the precipitation was carried out in a hot solution containing no appreciable amount of mineral acid, while Lingane and Kerlinger added 24 milliequivalents of hydrochloric acid before adding the pyridine to the cool solution. In Ostroumov's procedure the higher temperature is favorable for the decomposition of the complex of chromic ion with pyridine and the higher pH decreases the solubility of hydrous chromic oxide.

### EXPERIMENTAL

REAGENTS. Pyridine solution (1 to 2). Dissolve 50 ml. of pyridine in 100 ml. of water.

Sodium bisulfite, 20 per cent. Dissolve 2 grams of sodium bisulfite in 8 grams of water. Prepare a fresh solution daily.

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Table I. Coprecipitation of Chromium with Hydrous Ferric Oxide in Pyridine Separation

Experiment No.	Chromium in Sample Mg.	Ferric Iron in Sample Mg.	Chromium Remaining in Filtrate %
1	260 CrIII	500	0
2	390 CrIII	500	1.76
3	1.00 CrVI	279.2	<4
4	1.3 CrVI	279.2	10
5	2.6 CrVI	279.2	16
6	5.2 CrVI	279.2	19
7	15.6 CrVI	279.2	31
8	26.0 CrVI	279.2	36
9	52.0 CrVI	279.2	41
10	20.8 CrVI	0	100

**PYRIDINE SEPARATION, PROCEDURE I.** Transfer the solution containing no excess mineral acid to a 100-ml. volumetric flask, add 1 ml. of sulfuric acid (1 to 1) and dilute to about 80 ml. Add 15 ml. of pyridine (1 to 2) slowly while swirling, dilute to exactly 100 ml., and mix well. Filter through a dry rapid filter paper, such as Whatman No. 41, into a dry beaker. Transfer a 50-ml. aliquot to a clean 100-ml. volumetric flask and continue according to Polarographic Procedure I (1) to determine manganese as tri-dihydrogen pyrophosphatomanganate.

**PRECIPITATION OF CHROMIUM BY PYRIDINE.** In a series of experiments on the precipitation of chromic chromium in the absence of ferric iron it was found that if no appreciable amount of mineral acid was present when the pyridine was added, the pH of the solution became large enough so that hydrous chromic oxide was precipitated. However, the precipitate redissolved during 16 hours of contact with the pyridine solution, forming a rich green-colored complex with pyridine. When the final concentration of chromic ion exceeded approximately 0.01 *M*, the precipitation was incomplete. This probably was due to the increasing amount of free mineral acid liberated during the precipitation of hydrous chromic oxide. The pyridine separation, thus, does not seem to be satisfactory for the separation of chromic chromium in the absence of ferric iron or aluminum.

Table I shows the extent of coprecipitation of chromic chromium with ferric iron. The chromium content of the filtrate was determined polarographically. It is evident that as much as 260 mg. of chromic ion is quantitatively coprecipitated with 0.5 gram of ferric iron. This corresponds to a steel containing 34% chromium, a much larger percentage than is generally encountered.

That chromate is only partially coprecipitated with hydrous ferric oxide during the pyridine separation is shown by experiments 3 to 9, Table I. Chromium must be reduced to the trivalent state in order to be quantitatively coprecipitated. This is easily accomplished by adding a little sodium acid sulfite to the solution. The excess sulfur dioxide is removed by boiling.

**COPRECIPITATION OF VANADIUM WITH HYDROUS FERRIC OXIDE.** Using a sample containing 10 ml. of 0.1 *M* ammonium metavanadate alone, no precipitate formed at all when the Pyridine Separation Procedure I was carried out. The solution remained yellow in color.

Samples containing 0.279 gram of iron as ferric nitrate and from 0 to 76.4 mg. of vanadium as vanadate were prepared and the pyridine separation was then carried out. The filtrate was analyzed polarographically. No diffusion current due to vanadate was observed in any of the solutions containing up to 61.1 mg. of vanadium corresponding to an atomic ratio for vanadium to iron of 1.2 to 5. The qualitative hydrogen peroxide test also showed the absence of the vanadate ion. When the amount of vanadate was increased to 76.4 and 127.4 mg., the filtrate was yellow-colored. The percentage of vanadate in the filtrate was estimated colorimetrically to be 5 and 6%, respectively. It may be concluded that as much as 18% vanadium (as vanadate) in iron can be separated with hydrous ferric oxide during the pyridine separation.

Ferric vanadate is insoluble in neutral solutions but dissolves readily if a small amount of mineral acid is added to the solution. The above separation of vanadate is not, then, necessarily one of coprecipitation. However, if a simple precipitation of ferric vanadate occurred, one would expect a larger percentage of vanadate to be precipitated. The fact that this is not the case indicates that the vanadate is actually coprecipitated with the hydrous ferric oxide. The extent of coprecipitation is limited by the low pH at which the hydrous ferric oxide is formed in the pyridine buffer.

When the pyridine separation was carried out using a sample containing only 25.8 mg. of vanadium, as sodium vanadyl sulfate, a finely divided gray-green precipitate formed which was difficult to separate by filtration. By a polarographic determination, it was established that 92% of the vanadyl ion was precipitated.

Since vanadyl ion is partially precipitated even in the absence of iron, it should be largely coprecipitated with ferric iron. The pyridine separation was employed using samples containing 0.5 gram of iron as ferric nitrate and 5.1 to 127.4 mg. of vanadium as sodium vanadyl sulfate. No vanadyl ion was detected in the filtrate polarographically or colorimetrically. It is evident that the amount of vanadyl ion coprecipitated with ferric iron may even be greater than the amount of ferric iron precipitated.

**COPRECIPITATION OF CERIUM WITH HYDROUS FERRIC OXIDE.** The pyridine separation was employed using samples containing 14 to 140 mg. of cerium as ceric sulfate and 279 mg. of iron as ferric nitrate. No cerium was detected in the filtrate, showing that as much as 33% of cerium in steel can be coprecipitated during the pyridine separation, provided it is present as ceric ion.

However, if a reducing agent were added to reduce chromic ion and vanadium, any cerium would finally be present as cerous ion. Accordingly, the extent of the coprecipitation of cerous ion with hydrous ferric oxide was determined.

Samples containing 14 to 140 mg. of cerium as cerous sulfate and 279.2 mg. of iron as ferric nitrate were treated according to Pyridine Separation Procedure I and then analyzed polarographically. No cerium was found in the filtrate from samples containing 70 mg. or less of cerium. However, when the amount of cerium was increased to 140 mg., the cerium was no longer quantitatively coprecipitated. As much as 70 mg. of cerous cerium can be separated with 279.2 mg. of ferric iron during the pyridine separation. This corresponds to 20% of cerium and is a much larger percentage than is generally employed even in special cerium steels.

**COPRECIPITATION OF MANGANESE WITH HYDROUS FERRIC OXIDE.** If a 50-ml. aliquot of the pyridine filtrate is taken for the determination of manganese by Polarographic Procedure I (1), 2.5 ml. of pyridine remain in the sample to be determined. It was found that the pyridine introduced did not interfere with the formation of the violet tri-dihydrogen pyrophosphatomanganate ion. However, the average current per millimolar concentration of tri-dihydrogen pyrophosphatomanganate was 2.0% lower than the value obtained in the absence of pyridine.

To determine whether manganese was appreciably coprecipitated, a series of experiments (Table II) was performed using samples containing 0.8 gram of iron as ferric nitrate with various amounts of manganese as manganous sulfate. In experiments 7 through 12, 0.8 gram of pure iron was dissolved in 20 ml. of nitric acid (1 to 3) and the excess acid was neutralized with ammonium hydroxide. Pyridine Separation Procedure I and Polarographic Procedure I were then employed (1). To determine if manganese was lost by coprecipitation, a series of control experiments was carried out in the same manner, except that the manganese was not added until after the precipitation and separation of hydrous ferric oxide. Comparing the true diffusion currents obtained with the manganese added before and after the pyridine separation it may be observed that the current was within experimental error, the same. These results substantiate Ostroumov's (3) observation that the loss of manganese due to coprecipitation with hydrous ferric oxide by the pyridine method is extremely small.



BEHAVIOR OF FERROUS IRON IN PYRIDINE SEPARATION. Ferric iron may be partially reduced if sodium bisulfite is added to reduce chromate. The behavior of ferrous iron during the pyridine separation is not important in the polarographic procedure for the determination of tri-dihydrogen pyrophosphatomanganate but may be of importance if a quantitative separation of iron is desired. A sample containing 55.8 mg. of iron as ferrous sulfate yielded no precipitate at all when the pyridine separation was used in an atmosphere of carbon dioxide. To determine if ferrous iron is coprecipitated with ferric iron, the pyridine separation was employed using 10 ml. of 0.1 *N* ferrous sulfate solution and 5 ml. of 1 *M* ferric nitrate solution. The reduction of an aliquot of the filtrate with 0.1 *N* potassium dichromate showed that ferrous ion, like manganous ion, is not coprecipitated with hydrous ferric oxide during the pyridine separation. Any ferrous ion formed during the reduction with sodium bisulfite can be oxidized by boiling the solution which contains a little nitric acid.

SIMULTANEOUS SEPARATION OF IRON, CHROMIUM, VANADIUM, AND CERIUM FROM MANGANESE. It has been shown that the vanadyl, vanadate, ceric, cerous, and chromic ions are quantitatively precipitated with an excess of ferric iron during the pyridine separation of hydrous ferric oxide. Chromate, however, divides. In order to ensure the quantitative separation of these ions, the dichromate ion in particular, some reducing agent must be added. Addition of a little 20% sodium bisulfite solution instantly reduced the dichromate in a slightly acid solution. The vanadate was reduced upon warming the solution. The addition of sodium bisulfite, furthermore, is desirable to effect the reduction of any manganese dioxide which may have precipitated during the solution of the sample and aids in the removal of any excess of oxidizing agent added during the solution of the sample.

That the separation of chromium as well as vanadium and cerium with hydrous ferric oxide was quantitative if sodium bisulfite was used as a reducing agent was shown in the following experiment. A sample containing 5 ml. of 1 *M* ferric nitrate, 5 ml. of 0.05 *M* potassium bichromate, 5 ml. of 0.1 *M* ammonium vanadate, and 2 ml. of 0.1 *M* ceric sulfate was transferred to a 100-ml. volumetric flask. One milliliter of 20% sodium bisulfite was added and the solution was boiled for a few minutes. The pyridine separation was employed and the solution was then analyzed according to Polarographic Procedure I (1). Since the residual current of  $-0.07$  microampere was obtained at 0.1 volt *vs.* the saturated calomel electrode using capillary I the separation of chromium, vanadium, and cerium with hydrous ferric oxide was quantitative.

That the simultaneous separation of chromium, vanadium, and ferric iron does not result in loss of manganese was shown in a series of experiments using solutions corresponding to a chromium-cobalt steel. The following comprised the sample:

5 ml. of 1 *M*  $\text{Fe}(\text{NO}_3)_3$  containing 279.2 mg. or 60 to 77% of Fe  
5 ml. of 0.05 *M*  $\text{K}_2\text{Cr}_2\text{O}_7$  containing 26.0 mg. or 5 to 7% of Cr  
5 ml. of 0.1 *M*  $\text{NH}_4\text{VO}_3$  containing 25.5 mg. or 5 to 7% of V  
5 ml. of 0.1 *M*  $\text{Co}(\text{NO}_3)_2$  containing 29.5 mg. or 6 to 8% of Co  
Various volumes of 0.1 *M*  $\text{MnSO}_4$  containing 2.2 to 110 mg. or 0.6 to 23% of Mn

## Table II. Coprecipitation of Manganese with Hydrous Ferric Oxide

Pyridine Separation Procedure I and Tri-dihydrogen Pyrophosphatomanganate Polarographic Procedure I (1). Capillary I,  $m^{2/3}t^{1/6} = 314 \text{ mg.}^{2/3}\text{sec.}^{-1/2}$ , av.  $i_d$  per millimolar concentrated  $\text{Mn}^{III} = 1.51$  microamperes at +0.1 volt (S.C.E.)

Experiment No.	Final $\text{Mn}^{III}$ Millimolar	Time of Addition of Mn	Mn Taken Mg.	Mn Found Mg.
1	0.5	Before ppt.	2.75	2.73
2	0.5	After ppt.	2.75	2.77
3	2.0	Before ppt.	10.98	10.91
4	2.0	After ppt.	10.98	11.01
5	5.0	Before ppt.	27.46	27.73
6	5.0	After ppt.	27.46	27.76
7	0.5	Before ppt.	2.75	2.73
8	0.5	After ppt.	2.75	2.80
9	4.0	Before ppt.	21.97	21.82
10	4.0	After ppt.	21.97	21.80
11	5.0	Before ppt.	27.46	27.35
12	5.0	After ppt.	27.46	27.43

The sample was transferred to a 100-ml. volumetric flask and 5 ml. of nitric acid (1 to 3) were added. One milliliter of 20% sodium bisulfite was added and the solution was boiled several minutes to expel the sulfur dioxide and reoxidize any ferrous iron. After neutralizing the excess acid with ammonium hydroxide, Pyridine Separation Procedure I and Polarographic Procedure I were employed. The results in Table III show that no manganese within experimental error was lost due to coprecipitation.

Table III. Separation of Manganese from Hydrous Ferric Oxide and Interfering Elements

(Using samples corresponding to a chromium vanadium cobalt steel. Sodium acid sulfite reduction of chromium followed by Pyridine Separation Procedure I and Tri-dihydrogen Pyrophosphatomanganate Polarographic Procedure I, 1)

Final $\text{Mn}^{III}$ Millimolar	Mn Taken Mg.	Mn Found Mg.
0	2.20	2.18
0.2	2.20	2.18
0.5	5.49	5.47
1.0	10.1	10.0
2.0	20.2	20.4
5.0	54.9	55.2
10.0	109.8	109.4

## SUMMARY

Vanadyl, vanadate, ceric, cerous, and chromic ions are quantitatively coprecipitated with an excess of ferric iron when hydrous ferric oxide is precipitated by the use of the weak organic base, pyridine. Chromate, however, divides between the precipitate and filtrate. Manganous ion, as well as ferrous ion, remains quantitatively in the filtrate. Pyridine is well suited for separation of the interfering metals, vanadium, chromium, cerium, and a large excess of iron from manganese prior to the polarographic determination of manganese as tri-dihydrogen pyrophosphatomanganate.

The separation of hydrous chromic oxide in the absence of ferric iron is quantitative only under special conditions, because chromic ion forms a soluble green-colored complex ion with pyridine. However, in the presence of at least a twofold excess of ferric iron by weight, the coprecipitation of chromic ion with hydrous ferric oxide is quantitative. During the precipitation of hydrous ferric oxide, chromate ion in all proportions divides between the filtrate and precipitate. The loss of chromium in this way was prevented by the addition of a little sodium bisulfite solution followed by boiling with a little nitric acid to remove the excess sulfur dioxide and reoxidize any ferrous iron.

In the absence of ferric ion, vanadyl ion is incompletely separated as a finely divided gray-green precipitate. In the presence of even a smaller amount of ferric iron by weight the coprecipitation of vanadyl ion is quantitative. Vanadate ion is quantitatively coprecipitated with hydrous ferric oxide if the weight of ferric iron is over four times greater than that of vanadium.

Ceric ion is quantitatively coprecipitated with hydrous ferric oxide. Cerous ion likewise is quantitatively coprecipitated with hydrous ferric oxide, provided the weight of ferric iron is four times greater than that of the cerous ion.

The excellent separation of the hydrous oxides from manganous and ferrous ions is explained by the fact that the solution is weakly acidic during the precipitation and by the ability of pyridine to form complex ions with manganous ions as well as ferrous (cobaltous, and nickelous) ions.

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# Determination of Vitamin A and Carotenoids in Butterfat

## Spectroscopic Characteristics of Butterfat Fractions and Problems Involved in Biological Interpretations

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Results of tests made with modifications of the saponification and ether-extraction procedure (8) for determination of vitamin A in butterfat are reported, with a discussion and partial interpretation of the spectroscopic characteristics of such ether extracts.

**N**O SINGLE method for the direct spectroscopic determination of vitamin A in butterfat has been generally employed. A related application of spectroscopic methods (1) to the study of the vitamin A content of milk has been made by Dornbusch, Peterson, and Olson (3). However, little has been done in the study of errors involved with different butterfat samples or the correlation of spectroscopic data with biological assays.

In experiments in cooperation with the Technical Committee on Vitamin A Researches (8), a procedure of saponification and extraction with ether was developed. Details for spectrophotometric application have been reported (15). This paper reports the results of tests made with modifications of this procedure and presents a discussion and partial interpretation of the spectroscopic characteristics of such ether extracts. Attempts were made to correlate results calculated from direct spectroscopic data with those of the biological method in the case of samples (6, 7) which had been assayed by the rat-growth method.

### EXPERIMENTAL

Since  $\beta$ -carotene and vitamin A per se are responsible for practically the entire vitamin A potency of normal butter, the analytical problem is primarily the determination of these two compounds in the presence of related carotenes and carotenols. The carotene content is no indication of the vitamin A alcohol content; hence, for direct determination of vitamin A, ultraviolet spectrophotometry of an extract is necessary.

Clarification of butter samples consisted of filtration of the melted fat through filter paper at 55° C. during 2 to 3 hours, to remove water and salt.

Carotenoids were estimated from absorption values at 4370 and 4360 Å., which are the coincident points for the absorption curves of  $\beta$ - and neo- $\beta$ -carotenes in ether (15) and hexane (2), respectively. Wave lengths 4525 and 4675 Å. are of possible utility in the estimation of individual carotenoids (see Figures 1 and 2). They may be used with most spectrophotometers with a minimum possibility of error due to inexact wave-length calibration or to wide spectral regions isolated because the maximum of  $\beta$ -carotene occurs at 4525 Å. and the minimum of  $\beta$ -carotene at 4675 Å. [also near the flat region of the neo- $\beta$ -carotene curve (2)].

The nature of the carotenoids found in butter is dependent largely upon the feed of the cows, as pointed out by Strain (13) and others. In Figure 1 are representative characteristic curves of the total carotenoids found in the butters studied. Sample 106 was specially churned at the Purdue creamery. The standard curves of  $\beta$ -carotene and its isomer neo- $\beta$ -carotene (2) are included for reference. [This neo- $\beta$ -carotene fraction probably consists largely of the isomer designated by Polgár and Zechmeister (10) as neo- $\beta$ -carotene B.] All curves were placed to coincide at 4370 Å. It is noted that curve 8 is characterized by higher relative absorption than curve 7 in regions near 4000 and 4250 Å., which is in agreement with similar observations of Strain (13).

To obtain a characteristic absorption curve of the total carotenoids with a minimum amount of induced isomerization, a butter

sample from a cow fed a carrot-root carotene supplement was simply dissolved in ether, washed with water, dried with sodium sulfate, and filtered. The characteristic curve showed evidence of the presence of  $\alpha$ -carotene and was very close to absorption curves of the ether extract after saponification, in spite of solvent differences due to the large amount of fat present. In this experiment a 7-cm. thickness was studied spectroscopically by comparing an 8-cm. cell with a 1-cm. cell, both filled with the butterfat solution to avoid differences in apparent absorption due to refractivity differences.

Another butterfat, 106, was dissolved in hexane, and washed free of carotenols with 94% diacetone alcohol (2). The characteristic curve (Figure 2) of the resultant carotene fraction corresponded well between 4360 and 4950 Å. with that of a mixture of  $\beta$ -carotene and neo- $\beta$ -carotene. Upon analysis for these two components (2) the percentage of  $\beta$ -carotene was 77.1 at 4780 Å. and 79.1 at 4850 Å. Adsorption of this solution on columns of magnesia and alumina indicated the presence of two or more pigments. Figure 2 includes curves of the carotene fraction from each type of silage butter and from referee sample No. (15), all of which were prepared by evaporating the ether extract almost to dryness, dissolving in hexane, and finally washing with 92% aqueous methanol. All curves were placed to coincide at 4360 Å.

Vitamin A was estimated from the absorption value at 3240 Å. which is the absorption maximum of vitamin A alcohol in ether solution (15). The characteristic curves of Figure 3 were arbitrarily placed to coincide with the standard curve of vitamin A alcohol at 3400 Å. The carotenoid content of butterfats is subject to wide variation and carotenoids other than  $\beta$ -carotene are

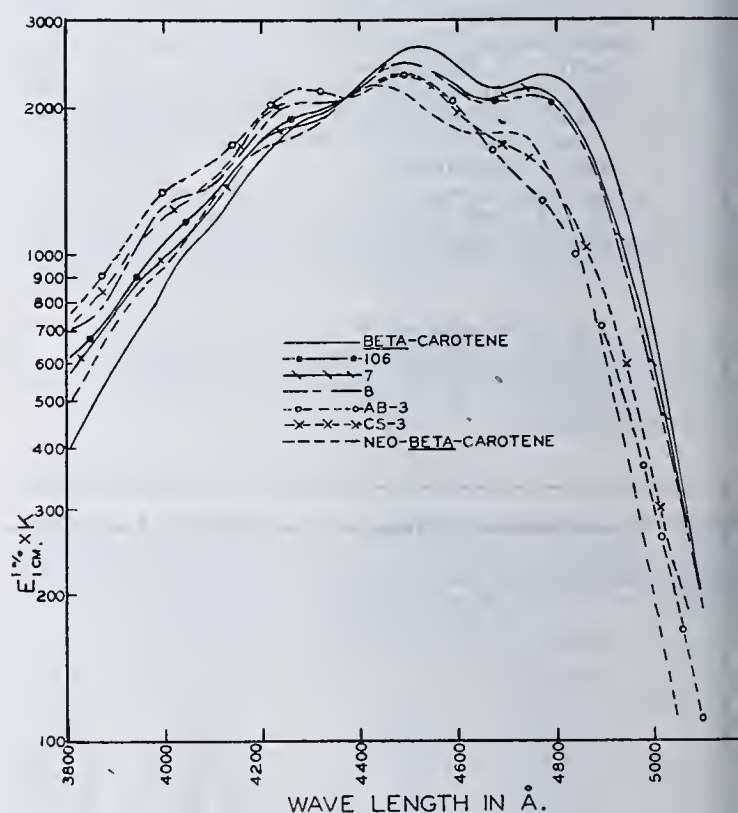


Figure 1. Absorption Spectra of Total Carotenoids in Ether Solution

- 106. Winter creamery butter, no artificial color added
- 7. Butter from cow fed alfalfa hay
- 8. Butter from cow fed carrot-root carotene supplement
- AB-3. Butter from cow fed alfalfa-brome silage
- CS-3. Butter from cow fed corn silage



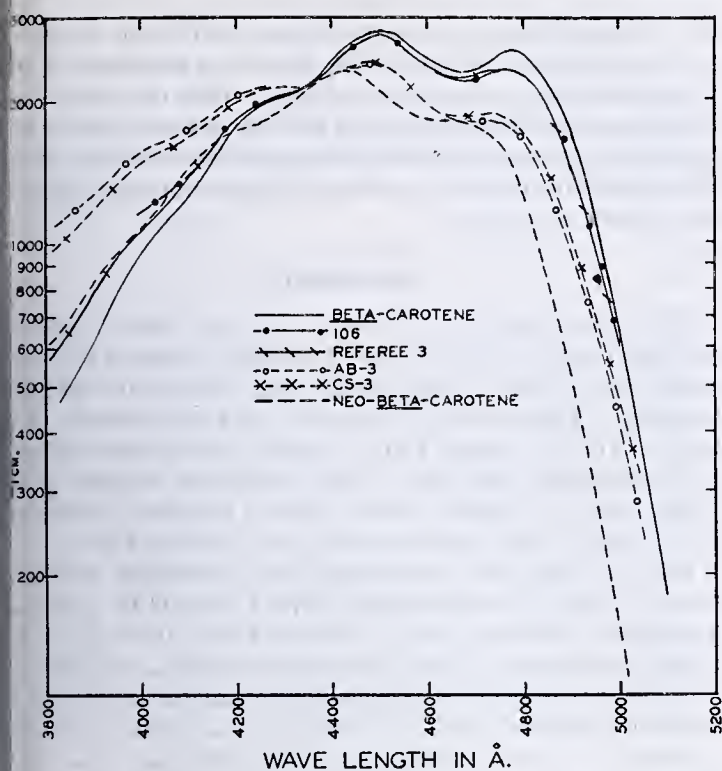


Figure 2. Absorption Spectra of Carotene Fractions in Hexane Solution

106. Winter creamery butter, no artificial color added  
 AB-3. Butter from cow fed alfalfa-brome silage  
 CS-3. Butter from cow fed corn silage

usually present. The ultraviolet absorption characteristics of such miscellaneous carotenoids have not been established. Colorless materials which absorb in the ultraviolet region probably also accompany vitamin A in the ether extract. Accurate corrections at 3240 Å. for absorption by carotenoids and other substances in such extracts are impossible at present. The necessity for some correction, even though it be somewhat arbitrary, is indicated by the curves of Figure 3, some of which agree very well with that of vitamin A below 3400 Å. They are invariably higher than the vitamin A curve at longer wave lengths, in part because of different carotenoid contents. Sample B3 was most colorless and was obtained from a cow fed on a ration lacking carotenoids but supplemented with vitamin A in the form of a h liver oil concentrate.

**RELIABILITY OF EXPERIMENTAL PROCEDURE (8, 15).** *Saponification Time.* A sample of commercial butter was clarified andaponified with aqueous methanolic potassium hydroxide in duplicate for 5, 10, 15, and 30 minutes. The eight absorption values of the ether extracts were constant to  $\pm 2.5\%$  at 3240 Å. and to  $\pm 1\%$  at 4500 Å. A 15-minute saponification with ethanolic instead of methanolic potassium hydroxide gave essentially the same result. Methanol is preferred because it is more stable with potassium hydroxide.

*Number of Ether Extractions.* Successive ether extracts were examined spectroscopically to determine their relative effectiveness in the removal of carotenoids and vitamin A. A rendered creamery butter with no artificial coloring matter was used. The first extract (100 ml.) removed 70% of the substance absorbing at 3240 Å. and about 91% of the carotenoids. The second extract (50 ml.) accounted for 20% additional absorption in the ultraviolet and removed all the remaining carotenoids. The third extract (50 ml.) accounted for 6.5% ultraviolet absorption and the fourth extract of 50 ml. left in the hypophase no appreciable quantity of material absorbing at 3240 Å.

*Effect of Ordinary Laboratory Illumination.* Commercial butter was clarified and analyzed in duplicate in two kinds of glassware. In one case, all manipulations were done in amber glassware (Kimble amber laboratory glassware), as recommended by Emery (4); in the other, ordinary Pyrex ware was used. Results of the two samples agreed within 1% in both the visible and ultraviolet regions, indicating that ordinary diffuse laboratory light does not affect the spectroscopic values obtained under these conditions. Absorption values of pure vitamin A in ethanol solution were not changed by fairly intense irradiation from an incandescent source (14).

*Sampling Error.* Many duplicate determinations made during this study indicate that butter is easily sampled after removal of water and salts. The surface butterfat exposed to air was avoided when possible. Spectroscopic results were easily duplicated with differences of 1% or less.

*Recovery of Added Vitamin A.* Two samples of pure vitamin A alcohol (0.325 mg.) were carried through the entire analytical procedure in the absence of butterfat. The resultant extract had a characteristic curve identical with that of vitamin A and a spectroscopic recovery of  $93 \pm 1\%$  was obtained. In another experiment, 3 mg. of crystalline vitamin A alcohol were dissolved in 30 grams of melted butterfat that was very low in carotenoids. One gram of this butterfat-vitamin mixture was then diluted tenfold with more melted butterfat. After analysis the absorption values of the butterfat sample alone were subtracted from those of the enriched sample (in duplicate). Characteristic absorption curves that agree well with those of pure vitamin A alcohol were thus obtained. Recoveries of 97.0 to 95.5% were calculated. A similar experiment was performed on duplicate samples of a butterfat (sample 7) rich in carotenoids. Exact duplication of the characteristic vitamin A curve was obtained in this case. Recoveries of 99.4 and 94.2% were calculated.

*Stability of Extracts.* The ether solutions from sample 7 were examined spectroscopically after 2 weeks in cold storage at 4° C. While no change, qualitative or quantitative, was detected in the region above 4000 Å., a general decrease in absorption was found below this wave length. This decrease amounted to 9% at 3240 Å. A decrease of similar magnitude in 30 hours was noted in the extract from a relatively "colorless" sample. These results are typical and indicate the necessity for prompt spectroscopic readings in the ultraviolet, whereas observations in the visible region may be considerably delayed if extracts are stored under the proper conditions.

**ATTEMPTS TO IMPROVE ULTRAVIOLET CHARACTERISTIC CURVES.** Attempts were made to reduce the general absorption in the ultraviolet by purification of the vitamin A extract through removal of interfering substances.

*Clarification.* The authors' observations indicate that spectroscopic data on butters in the visible region may be converted to the butterfat basis by multiplying by the factor 1.20, as calculated from carotenoid absorption in the visible region. This is presumably due only to the removal of water and salt as colorless material by clarification. Clarification also removes certain materials which absorb in the ultraviolet, and the corresponding correction factor at 3240 Å. was ca. 1.35 for the three samples examined. It is advisable to clarify samples of butter before analysis because such materials may not be uniform in all butters and because the analytical data are thus placed on the fat basis, making variations in water or salt content unimportant.

*Adsorption.* When the ether extract was passed through a 10-cm. (4-inch) column of magnesia-Supercel (50-50) and the ether percolate made to volume, no change was noted in the absorption spectrum of the carotenoids in the visible region, or in the quantity of total carotenoids present. The ultraviolet absorption, however, increased about 10% at 3240 Å.

Treatment of a "colorless" butterfat with Lloyd's reagent, which has been used to decolorize butters and remove vitamin A (12), failed to provide a base curve which could be interpreted as representative of the general absorption—i.e., other than that represented by vitamin A. The difference between the butterfat curve and that of decolorized fat did not approach the curve of vitamin A.

*Acid Extraction.* When the final ether solution from sample 106 was extracted with 0.5 N hydrochloric acid, followed by 0.5 N sodium hydroxide and water, a small decrease in ultraviolet absorption occurred and small changes (perhaps isomeric) were found in the visible absorption.

*Freezing.* The ether extract was chilled in a dry ice-acetone bath and rapidly filtered on a cold filter in an effort to freeze out impurities. A 14% decrease occurred in the absorption value at 3240 Å. but the decrease was general in the ultraviolet and, therefore, no improvement in the characteristic curve resulted.

**STABILITY OF CAROTENOIDS IN STORED SAMPLES.** Ten months after the first spectroscopic observations, referee samples 6 (15) and 7 (both kept at  $-20^{\circ}\text{C}$ .) were re-examined. For the former sample, the 4370 Å. absorption remained constant but the value at 4780 Å. decreased 5%. In the case of sample 7, however, the absorption at 4370 Å. decreased 5% but that at 4780 Å. decreased 28% and the resultant characteristic curves of the total carotenoids and the carotene fraction from this sample now resembled



those from CS-3, a silage butter. The cause of these changes was not investigated, but it is evident that the carotenoids in butterfat may undergo considerable change with time, even at low storage temperatures. Isomerization may be involved.

**CORRELATION BETWEEN SPECTROSCOPIC AND BIOLOGICAL RESULTS.** Twenty-four samples were assayed biologically by the rat-growth method. Fourteen relatively "colorless" samples were produced from two cows fed rations lacking in carotenoids but supplemented by various amounts of vitamin A in the form of a fish liver oil concentrate. Ten samples of yellow butter were from cows fed alfalfa hay or a diet supplemented by various amounts of carrot-root carotene (7). All samples were stored at  $-20^{\circ}\text{C}$ . Unfortunately, the time intervals (1 to 21 months) were rather great between the dates of churning and the spectroscopic observations.

The vitamin A activity of the relatively "colorless" samples is attributed almost exclusively to vitamin A *per se*, derived from cod liver oil of the ration supplement. Corresponding values of  $E_{1\text{ cm.}}^{1\%}$  (observed on unclarified butters) were corrected for water and salt content by multiplication of the observed value by 1.35 and for general absorption by 0.78, a factor discussed previously (15). The vitamin A contents corresponding to such absorption values were calculated on the assumption that the corrected absorption in the ultraviolet was due to vitamin A alcohol ( $E_{1\text{ cm.}}^{1\%} = 1825$  at  $3240\text{ \AA}$ ).

Most of the samples high in carotenoids were clarified before analysis; for such samples the ultraviolet absorption values were simply multiplied by the factor 0.78 before calculation of vitamin A content, and total carotenoids were determined from absorption measurements at  $4370\text{ \AA}$ . (15). The total carotenoid content was divided by 2 (9) and added to the vitamin A content.

With neither set of butters were spectroscopic calculations sufficiently well correlated with biological results to establish a clear cut relationship. When a line was drawn through the ori-

gin to approximate the median line, individual samples of "colorless" butters deviated from this line a maximum of 20% with a mean absolute deviation of 10%. With the yellow series it was apparent that the two very different types of rations make a separate treatment desirable with regard to correction factors. Deviations from median lines similar to those from the "colorless" series could be obtained.

## DISCUSSION

The varied nature of the curves for total carotenoids from different types of butter (Figure 1) and the changes which carotenoids may undergo during storage make difficult the optical estimation of the particular carotenoids that have vitamin A potency. The wave length  $4370\text{ \AA}$ . appears to be the best choice for total carotenoid estimation, at least until more detailed spectroscopic data are available on the various individual carotenoids present and on such of their isomers as may occur in butter.

It is reasonable that a measure of total carotenoids, such as obtained by the use of wave length  $4370\text{ \AA}$ ., should be inadequate in itself and should require a correction factor, the magnitude of which is dependent on the dietary source of the carotenoids.

Carrot-root carotene contains large amounts of  $\alpha$ -carotene, and some  $\gamma$ -carotene in addition to  $\beta$ -carotene, as well as appreciable quantities of other carotenoids, while alfalfa hay contains  $\beta$ -carotene, various carotenols, and decomposition products of carotenoids. Recent developments in the application of isomerization methods to the study of carotenoids (2, 10) show the complexity of this problem.

The rather high content of carotenols found in the referee butters (15) causes the carotene fraction to be considerably more significant than the total carotenoids from the standpoint of measurement of vitamin A potency. It is possible that measurements on the carotene fraction would have required smaller correction factors than were applied to the total carotenoid absorption.

It is noted in Figure 2 that the curves for sample 106 and referee sample 3, creamery butters of January and July, respectively, are very close together and approximate the curve of a mixture of  $\beta$ -carotene and neo- $\beta$ -carotene. Indeed, the characteristic curves for all six referee samples, taken at intervals throughout the year, were remarkably similar in both the ultraviolet and visible regions. Clarification and hot saponification may promote isomerization of  $\beta$ -carotene but it is not unlikely that neo- $\beta$ -carotenes occur naturally in butterfat. Strain reported no spectroscopic change in the carotenoid spectrum after heating 3 hours at  $50^{\circ}\text{C}$ . during clarification (13). It is evident that the carotene fraction of butters from silage-fed cows is qualitatively different from those of butters from other sources in agreement with previous work on this subject (11).

Ultraviolet characteristic curves of ether extracts from some butterfats show a decreasing (or at least not greatly increasing) absorption below  $3240\text{ \AA}$ . Most of the interfering absorption in this region may be due to carotenoids. Removal of the carotenoid fraction from referee sample 3 by 90% methanol decreased the ultraviolet absorption very much. However, the ultraviolet characteristic curve of the carotenol fraction (after transfer to ether) did not resemble that of vitamin A any more closely than did the curve of the original ether extract.

Numerous other methods of handling the data were tried in an attempt to obtain a straight-line relationship which would result in smaller deviations, but no better results were obtained. In the case of the butters of the yellow series, the carotenoids were treated as a mixture of  $\beta$ -carotene and neo- $\beta$ -carotene. Addition of the calculated  $\beta$ -carotene content (after division by 2) to the vitamin A content gave no better correlation. The maximum deviations for these samples are smaller than those reported by Fraps, Kemmerer, and Meinke (5), who applied corrections of somewhat similar nature to butters.

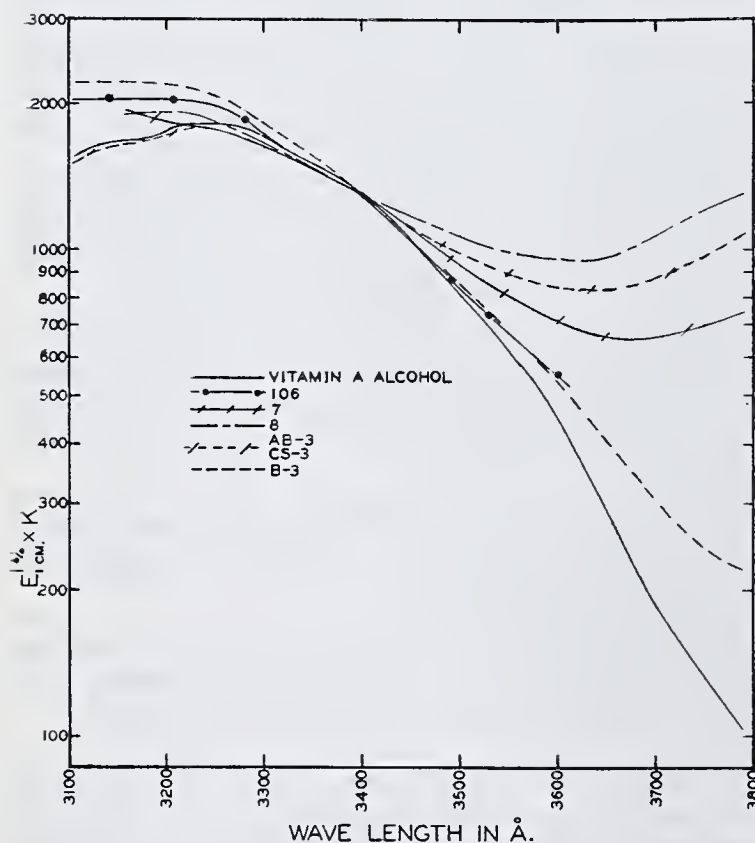


Figure 3. Ultraviolet Absorption Spectra of Ether Extracts

- 106. Winter creamery butter, no artificial color added
- 7. Butter from cow fed alfalfa hay
- 8. Butter from cow fed carrot-root carotene supplement
- AB-3. Butter from cow fed alfalfa-brome silage
- CS-3. Butter from cow fed corn silage
- B-3. "Colorless" butter from cow fed on ration lacking carotenoids



Efforts to obtain agreement between spectroscopic and biological results with six silage butters failed completely. The pigment system in such butters is too complex to permit a similar interpretation at the present time. This is recognized as a serious objection to the use of this method at present. Considerable improvement is needed before this method can be successfully employed on butterfats from cows on different diets, even for comparative purposes.

The calculation method presented above was based on numerous assumptions which are not necessarily better than others that might well be made. The necessity for such assumptions arises from the great complexity of the problem and the multitude of factors which interfere with a direct and simple spectrophotometric determination of vitamin A-potent substances in butterfats. Errors inherent in the biological method must also be considered in such correlations as were attempted here. An exact statistical treatment of the data was not considered profitable because of the many sources of error involved. It is hoped that these observations may be of assistance in the final development of a more direct treatment and more general application of absorption data to obtain contents of vitamin A-potent substances in butterfats. The extraction procedure employed is considered adequate and reliable. Further fractionation appears desirable, especially the separation of vitamin A from other carotenoids.

#### SUMMARY

Samples of butterfat produced under different dietary conditions were studied by the direct spectroscopic method. Total carotenoids were estimated and ultraviolet measurements were made on the unsaponifiable fraction. Characteristic curves of the total carotenoids and of the carotene fraction from very light "colorless" butters, yellow butters, and butters from cows fed alfalfa-brome grass and corn silages, were compared with that of carotene. Corresponding curves of the unsaponifiable fraction in the ultraviolet region were compared with that of vitamin A. Effects of clarification, adsorption, acid extraction, and freezing upon the characteristic curves were studied, as well as various factors affecting the reliability of the experimental procedures.

Twenty-four samples were assayed biologically and attempts made to correlate spectroscopic with biological values. No clear-cut relationship could be established. The feed of the cows has a great influence on the nature of the carotenoids present in the butterfat. More extensive purification of the vitamin A fraction is desirable for the successful application of direct spectrophotometry to the determination of vitamin A in butterfats.

#### ACKNOWLEDGMENT

The writers are grateful to J. G. Baxter, Distillation Products, Inc., for the sample of crystalline vitamin A for use as a standard, R. E. Roberts, Purdue University Creamery, for sample 106, and C. J. Koehn for use of referee samples.

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## Adaptor for Angle Centrifuge Tests

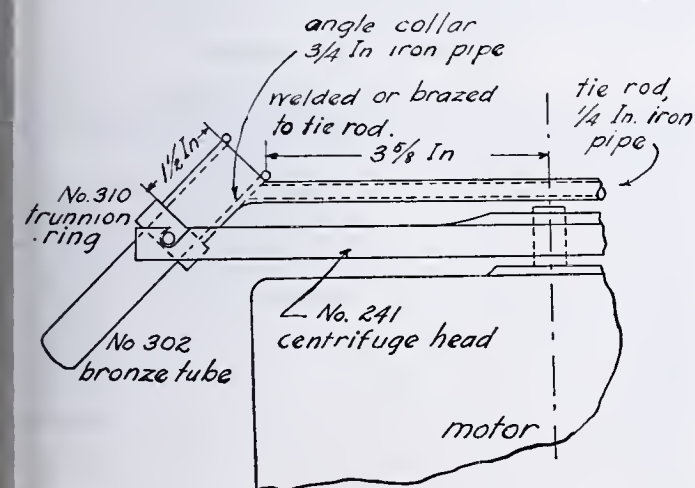
W. E. BAIER, California Fruit Growers Exchange, Research Department, Ontario, Calif.

A COMMONLY used laboratory centrifuge is the International, usually fitted with tubes or cups swinging on trunnions. Other types hold the tubes in some fixed inclination relative to the axis of rotation. The patented angle centrifuge as

supplied by Ivan Sorvall employs angles up to 50° and for certain classes of work offers advantages of speed and efficiency as compared with the trunnion cup type. The International Equipment Co. offers interchangeable angle heads.

Any laboratory already equipped with an ordinary centrifuge may readily improvise the means for testing the efficacy of the angle centrifuge in some particular application and thereby justify the investment in the second machine. Under present conditions the improvised equipment may well suffice for regular operation where a two-tube angle centrifuge is adequate. In some work it is advantageous after angle centrifuging to transfer the tubes to the ordinary pivoted trunnions, thus to compact a precipitate at the bottom of the centrifuge tube. Routine simultaneous treatment by pairs in this manner is conveniently accomplished with the present device.

The sketch shows the construction of a simple adaptor which was made for conducting tests with a size 2 International centrifuge, No. 241 centrifuge head, No. 310 trunnion rings, and No. 302 bronze tubes. When the adaptor is rested upon the two opposite trunnion rings and the No. 302 bronze tubes are inserted in the collars and trunnion rings, the system becomes rigidly positioned and is ready for operation as an angle centrifuge.





# Titrimetric Determination of Zinc

## Application to Alloy Analysis

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The purpose of the work described was to develop a rapid control method for the determination of zinc in alloys and ores. The procedure consists of precipitation of zinc as the oxalate in aqueous acetic acid solution, centrifugal separation of the precipitate, solution of the precipitate in sulfuric acid, and titrimetric determination with standard permanganate solution of the oxalic acid formed. The application of the method to alloys and concentrates is described.

THE principal purpose of the work described in this paper was to develop a rapid control method for the determination of zinc in alloys, which might be used in place of the phosphate method. It was believed advisable to determine zinc in its customary position in the procedures usually used for alloy analysis. There has been no attempt to change the methods of determination of the other elements; only an attempt to simplify and shorten the zinc determination.

Since oxalate precipitates have two very desirable properties—namely, ease of ignition and applicability to permanganate titrations—the possibility of obtaining a zinc oxalate precipitate was investigated. The simplicity and speed of the titration of oxalic acid by permanganate solution were the factors which led to the choice of such a titration as a rapid method for the final determination of the zinc. A study was also made of the use of the centrifuge as an aid in the separation and washing of the zinc oxalate precipitate. The technique of the method as finally developed was largely based on that used by Elving and Caley (6) for the determination of magnesium.

The most important methods used for the separation of zinc are probably the precipitation of the zinc as the ammonium phosphate or as the sulfide.

The precipitation of zinc as the ammonium phosphate,  $\text{Zn} \cdot \text{NH}_4\text{PO}_4$ , is accomplished by adding a large excess of diammonium hydrogen phosphate to a neutral solution containing a high concentration of ammonium salts. The zinc ammonium phosphate precipitate can be weighed as such by drying at  $105^\circ$  to  $110^\circ \text{C}$ ., or it may be changed to zinc pyrophosphate,  $\text{Zn}_2\text{P}_2\text{O}_7$ , by ignition at  $900^\circ \text{C}$ . This method is used for the determination of zinc in brasses and bronzes. The procedure for analysis requires that the tin, lead, and copper be first removed as stannic oxide, lead oxide or sulfate, and copper. If iron is present, it is precipitated as the hydroxide and filtered off. Zinc is then determined in the filtrate.

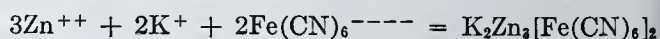
The precipitation of zinc sulfide is very often used in the analysis of ores. Zinc can be separated from aluminum, iron, cobalt, nickel, manganese, and chromium by saturating a buffered solution of the ions with hydrogen sulfide. The solution must have a pH ranging from 2 to 3; several buffering systems have been proposed for obtaining this hydrogen ion concentration (5, 7, 9–12). The sulfide precipitate may be converted to the oxide by ignition at  $800^\circ$  to  $900^\circ \text{C}$ ., to the sulfate by dissolving in hydrochloric acid and evaporating with sulfuric acid, or to the pyrophosphate by solution and precipitation. It may be weighed as zinc sulfide after special treatment or its zinc content may be determined titrimetrically as subsequently described.

The electrolytic separation of zinc is best accomplished in a solution which has been made slightly acid with acetic acid and contains a considerable amount of sodium acetate. Alkaline solutions tend to give high results, due to the presence of zinc oxide or hydroxide deposit. The electrolytic methods, because of the special equipment needed, the experience and care necessary to get reliable results, and the unavoidable errors involved,

are often less desirable than the sulfide or the ferrocyanide method.

The most commonly used titrimetric method for the determination of zinc is the one in which a standard solution of potassium ferrocyanide is used as the titrant.

The zinc is first separated from the other interfering ions in solution by precipitation with hydrogen sulfide. The sulfide precipitate is dissolved in dilute hydrochloric acid, and hydrogen sulfide is expelled by boiling. The solution is made neutral with ammonium hydroxide and then acid again with dilute hydrochloric acid. After addition of ferrous sulfate solution it is titrated with a standard potassium ferrocyanide solution. Zinc is precipitated according to the following reaction:



Several indicators have been employed for this titration. Uranyl nitrate, ammonium molybdate, or ferric chloride is often employed as external indicator. The preferable internal indicators are diphenylamine, diphenyl benzidine, or sodium phenylamine sulfonate.

In connection with their determination, several elements have been precipitated as the oxalate. Calcium is almost universally determined in limestones and cements by the precipitation of calcium oxalate in an ammoniacal or acetic acid-buffered solution. In an aqueous medium a great many metallic oxalates appear to form complex oxalate ions which can be broken up with the resulting precipitation of the metallic oxalate by the addition of a relatively large volume of acetic acid (3, 4, 6, 13, 14, 15).

Zinc oxalate was precipitated by Classen (4) in the following manner:

A large excess of potassium oxalate and an equal volume of acetic acid were added to the solution containing zinc, iron, and aluminum nitrates. After standing at about  $50^\circ \text{C}$ . for 6 hours the precipitate was filtered and washed with a solution containing equal volumes of acetic acid, ethyl alcohol, and water. The zinc oxalate was converted to zinc oxide by heating for several minutes at red heat. The oxide was weighed. Nass (13) repeated Classen's work.

Ward (15) precipitated zinc oxalate from an acetate solution by adding 2 grams of oxalic acid and a volume of acetic acid equivalent to the volume of zinc acetate solution present. The solution was allowed to stand overnight and was then filtered through a mat of asbestos on a Gooch crucible. The zinc oxalate was dissolved in dilute (1 to 4) sulfuric acid and the resulting solution was then titrated with potassium permanganate solution.

In these studies the necessary conditions for successful precipitation were not sufficiently studied and the practical application of the method was not described.

### REAGENTS AND APPARATUS

**REAGENTS.** Baker's c.p. acetic acid was used; its purity was found by evaporating 50 ml. of the acid in a platinum dish. One-tenth of a milligram of residue was found after ignition in a muffle furnace at  $550^\circ \text{C}$ .

Standard zinc solutions containing 0.2008 and 0.999 mg. of zinc per ml. were prepared by dissolving the proper amount of Mallinckrodt's analytical reagent grade zinc in dilute sulfuric acid. Each solution was checked by evaporating a definite volume in a platinum dish. The residue was ignited at  $550^\circ \text{C}$ . and after cooling was weighed as zinc sulfate. A saturated solution of ammonium oxalate was prepared by dissolving ammonium oxalate in hot water and allowing the excess oxalate to crystallize on cooling.

**APPARATUS.** The steam bath used for digestion of the oxalate precipitate consisted of a large steam cone which was covered with a metal sheet with holes cut in it of such size as to allow much of the pear-shaped centrifuge tube as possible to be

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Table I. Effect of One Gram of Salt on Precipitation of Zinc Oxalate in a 70% Acetic Acid Solution

Salt	Zinc Present Mg.	Zinc Found Mg.	Difference Mg.
NH <sub>4</sub> NO <sub>3</sub>	10.0	10.0	0.0
NH <sub>4</sub> NO <sub>2</sub>	10.0	9.9	-0.1
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	10.0	10.0	0.0
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	10.0	9.9	-0.1
NH <sub>4</sub> Cl	10.0	0.0	a

No precipitate formed after 1.5 hours of digestion. After 12 hours some precipitate appeared.

Table II. Determination of Zinc by Titration of Oxalate with Potassium Permanganate

(Zinc oxalate precipitated from a 70% acetic acid medium)

Zinc Taken Mg.	Zinc Found Mg.	Difference Mg.
1.0	0.8	-0.2
	0.6	-0.4
	0.8	-0.2
	0.8	-0.2
10.0	9.9	-0.1
	9.9	-0.1
	10.0	0.0
	10.0	0.0
25.0	24.9	-0.1
	24.9	-0.1
	25.0	0.0
	25.0	0.0

used to the steam. An ordinary metal can or box would serve well if steam could be passed into it. The centrifuge used was a Precision Scientific Company model of the type commonly used for the determination of water and sediment in petroleum products (2). The tubes, which were usually employed for the same determination, had a pear-shaped bulb with a narrow stem and were of 100-ml. capacity. Any type of centrifuge tube, large enough and of construction to allow the precipitate to be slung to the bottom without being disturbed, might have been used. A straight-walled centrifuge tube of 100-ml. capacity with a tapering stem of the type also used for the determination of water and sediment in petroleum products (2) might avoid the difficulty occasionally encountered when the precipitate clings to the slanting walls of the pear-shaped tube. Pyrex all-glass wash bottles were used for washing the zinc oxalate precipitates. The removal of the centrifugate was accomplished by immersing a tube, which was connected to an aspirator through a series of two traps, in the supernatant liquid. This tube was drawn to a long tapering point, so that it could be immersed in the centrifuge tube without touching the sides.

#### PRECIPITATION OF ZINC OXALATE

EFFECT OF ACETIC ACID CONCENTRATION. The precipitation of zinc oxalate was studied in the following manner:

A known amount of standard zinc solution, containing 1, 10, or 25 mg. of zinc, was transferred to a 250-ml. beaker. Enough water was added to make the final volume, after the addition of acetic acid and oxalate solution, the desired concentration. For example, 10 ml. of standard solution which contained 1 mg. of zinc per ml. were pipetted into a 250-ml. beaker; then 15 ml. of water were added; next 70 ml. of glacial acetic acid; and finally 10 ml. of saturated ammonium oxalate solution were added dropwise with constant stirring. This gave an acetic acid concentration of 70%.

The precipitate was allowed to digest on a steam bath for 1 to 2 hours. The time required for digestion decreased with increasing acetic acid concentration. After digestion was completed, the solution was filtered through Whatman No. 42 filter paper and the precipitate was washed with an acetic acid solution of the same concentration as the medium in which the precipitation was carried out. A rubber policeman which had been soaked in glacial acetic acid for 3 days was used as an aid in transferring the precipitate to the filter paper. The paper and precipitate were placed in a weighed porcelain crucible and ignited at 650° C. The zinc was then weighed as the oxide. The results obtained indicated that the optimum concentration of acetic acid is 70%. At this concentration precipitation was complete, the precipitate agglutinated rapidly, and the solution was easily filtered.

EFFECT OF AMMONIUM SALTS. After the optimum acetic acid concentration had been determined, a study was made of the

effect of certain ammonium salts on the precipitation of zinc oxalate (Table I).

One gram of ammonium chloride, ammonium nitrate, or ammonium sulfate was added to 95 ml. of a solution containing 70 ml. of acetic acid and 10 mg. of zinc, and 5 ml. of saturated ammonium oxalate were then added with constant stirring. The precipitate was digested, filtered, washed, and ignited in the same manner as in determining the optimum concentration of acetic acid.

Apparently, ammonium sulfate and ammonium nitrate have no effect upon the precipitation of zinc oxalate. Ammonium chloride evidently inhibited precipitation, for no precipitate was obtained after 3 hours of digestion. However, some precipitate was obtained when the solution was allowed to digest overnight.

INDIRECT TITRIMETRIC DETERMINATION OF ZINC. Since most oxalates can be changed upon acidification to oxalic acid which can be titrated with standard permanganate solution, it was deemed advisable to titrate the oxalic acid formed by treating the zinc oxalate with sulfuric acid. The procedure finally evolved for the titrimetric determination of zinc was as follows:

The zinc solution is transferred to a 100-ml. centrifuge tube of the type described, 70 ml. of glacial acetic acid are added with enough water to make the final volume 95 ml., and 5 ml. of saturated ammonium oxalate solution are then added with stirring. The precipitate is allowed to digest on a steam bath for 1.0 to 1.5 hours. When digestion is complete, the tubes are placed in a centrifuge and rotated rapidly for 3 or 4 minutes. When the precipitate has been completely slung to the bottom of the tube, the centrifugate is removed by suction. Care is necessary, since some of the precipitate might be removed with the liquid. Any precipitate remaining on the sides of the tube can be removed by rubbing the side with a rubber policeman. The loosened precipitate is slung to the bottom as before.

The precipitate is loosened from the bottom of the tube and washed by spraying a stream of 70% acetic acid on it. This is best done by tipping the tube in such a way that the supernatant liquid drains from the precipitate. The washing is done in such a manner that all the precipitate is removed from the bottom of the tube and dispersed throughout the washing medium. Between washings the tube is centrifuged and the supernatant liquid is removed by suction. Occasionally it may be advisable to digest the precipitate for 10 minutes on the steam bath before centrifuging. When washing is completed (two washings are usually enough), the precipitate is dissolved in 25 ml. of dilute sulfuric acid (5 + 95). The solution is transferred to a beaker and the tube is rinsed three times with portions of the dilute sulfuric acid. The final volume is made up to 65 ml. The solution is titrated with 0.025 N potassium permanganate solution according to the procedure recommended by Fowler and Bright (8).

The results obtained by using this procedure with varying amounts of zinc present appear in Table II. This method is both rapid and accurate if the necessary precautions are observed.

An alternative method of separating the zinc oxalate would be to carry out the precipitation and digestion in a beaker, to filter and wash the precipitate on paper or a filtering crucible, and to dissolve the washed precipitate in warm or hot sulfuric acid (5 + 95), the solution obtained being used for the titrimetric determination of oxalate. Such a procedure would in many cases be preferable to the centrifugal separation as regards speed and convenience.

#### INTERFERENCES IN PRECIPITATION OF ZINC OXALATE

In order to determine the ions whose oxalates might precipitate in a 70% acetic acid solution, several qualitative tests were made with varying concentrations of several ions.

Standard solutions containing approximately 1 and 10 mg. per ml. were used. A definite amount, 0.1, 0.5, 1, or 10 mg., was added to a test tube. Enough water was added to make the volume 2.5 ml. Then 7 ml. of acetic acid and 0.5 ml. of saturated ammonium oxalate solution were added. If no precipitate appeared, the solution was heated in a hot water bath to determine whether precipitation would occur upon digestion. In case a precipitate appeared, the test was made again in the same man-



Table III. Qualitative Precipitation of Oxalates in 70% Acetic Acid

Ion Tested	Mg. <sup>a</sup>	NH <sub>4</sub> OH Addition	Result
Sn <sup>++++</sup>	1.0	No	No ppt.
Cu <sup>++</sup>	0.5	No	Light blue ppt.
	1.0	No	Light blue ppt.
	1.0	Yes	No ppt.
Pb <sup>++</sup>	0.1	No	No ppt.
	0.5	No	White ppt.
	0.5	Yes	White ppt.
Fe <sup>+++</sup>	10.0	No	No ppt.
Al <sup>+++</sup>	25.0	No	No ppt.
Mn <sup>++</sup>	0.1	No	Light white ppt.
	0.1	Yes	No ppt.
	0.5	Yes	Light white ppt.
Ni <sup>++</sup>	0.1	No	No ppt.
	0.5	No	Light green ppt.
	0.5	Yes	No ppt.
	1.0	No	Light green ppt.
	1.0	Yes	Light green ppt.

<sup>a</sup> Final volume, 10 ml.Table IV. Composition of National Bureau of Standards Samples<sup>a</sup>

No.	Alloy	Cu %	Zn %	Sn %	Pb %	Fe %	Ni %	Others %
37b	Sheet brass	70.36	27.09	0.99	0.90	0.21	0.45	...
52	Cast bronze	88.33	1.89	7.90	1.52	0.12	0.13	Sb 0.16
62	Manganese bronze	59.07	35.06	0.82	0.56	0.13	0.64	Mn 1.59, Al 1.13
63	Phosphor bronze	78.05	0.48	9.91	9.74	0.27	..	Sb 0.55, As 0.19, P 0.62

<sup>a</sup> Analyses obtained from National Bureau of Standards.

ner, except that the solution was made basic with ammonium hydroxide before adding acetic acid. The purpose of adding ammonium hydroxide was to attempt to tie up the ions in the form of ammonium complexes and to determine whether the oxalate would precipitate in the presence of the ammonium salt. The results appear in Table III.

Nickel, lead, and copper interfere when present in concentrations of 0.5 mg. or more in 10 ml. of the 70% acetic acid solution. Lead and copper are usually removed previous to the determination of zinc in a copper-base alloy and should cause no particular trouble. Nickel, however, is not removed by the usual procedure. Ammonium hydroxide added to the solution before the addition of acetic acid tended to prevent precipitation of the nickel oxalate. In order to determine if the nickel interference could be avoided, quantitative measurements were made by precipitating 10 mg. of zinc as the oxalate in the presence of 1 mg. of nickel ion and 1 mg. of ferric ion. The ferric ion was added because it is not usually removed from the solution before the determination of zinc. Iron, however, could be removed from solution by the addition of ammonium hydroxide and filtration of the ferric hydroxide. If the iron was to be determined this step would seem advisable. The results of these experiments were high by several tenths of a milligram, although results presented below show that the determination of zinc by the oxalate method in alloys containing up to 0.65% of nickel is satisfactory. If enough nickel is present to disturb the zinc determination, the nickel should be first precipitated and determined as the dimethylglyoxime derivative. When dealing with a sample containing an unknown amount of nickel this procedure is to be recommended. Manganese, if present in appreciable amounts, should be removed. In the complete analysis of an alloy it can be removed with the R<sub>2</sub>O<sub>3</sub> group after oxidation with bromine or persulfate to ensure its complete precipitation. If manganese is not removed, it will be precipitated along with the zinc and the value obtained for zinc will have to be corrected on the basis of the manganese present as determined by a colorimetric method applied to the original sample. If the zinc oxalate precipitate is determined by conversion upon ignition to the oxide, the manganese in the residue can be determined colorimetrically.

Interference is to be expected from the presence of any elements such as calcium and magnesium, whose oxalates are insoluble in 70% acetic acid solution.

## DETERMINATION OF ZINC IN BRASSES AND BRONZES

Zinc was determined by the oxalate method in four alloys issued as standard samples by the National Bureau of Standards. Two samples, sheet brass 37b and manganese bronze 62, contained high percentage of zinc; whereas two other samples, cast bronze 52 and phosphor bronze 63, contained less than 2.00% zinc. The certified analysis of the samples used appears in Table IV.

Tin, antimony, lead, and copper were taken out of solution according to the methods adopted by the American Society for Testing Materials (1). Platinum electrodes of the gauze type were used for the electrodeposition of the copper. The anode was rotated and the cathode remained stationary. Electrolysis was done on a Waco electrolytic apparatus, manufactured by the Wilkens-Anderson Company.

Several variations of the following procedure were tried, but the procedure given seems to be best.

**SHEET BRASS AND MANGANESE BRONZE.** Samples of approximately 0.5 gram are used. The alloy is dissolved in 10 ml. of concentrated nitric acid in a 200-ml. porcelain casserole and the solution is carefully evaporated to dryness. The residue is baked for a few minutes and then dissolved in 10 ml. of concentrated nitric acid. The volume of the solution is brought to 50 ml. by adding boiling water. The precipitate is digested near the boiling point for one hour, filtered, and thoroughly washed with hot water. The filtrate, in a 200-ml. electrolytic beaker, is electrolyzed with an initial current of 0.2 ampere and a potential between 1.8 and 2.0 volts. After electrolysis has been carried on for about 10 minutes one drop of 0.1 N hydrochloric acid is added to the solution. After 30 minutes, electrolysis is completed and most of the lead deposited on the anode, 3 ml. of concentrated sulfuric acid are added and the current is increased to 0.4 to 0.5 ampere. Voltage should not exceed 2.1 volts.

After allowing ample time for the copper to be completely separated, usually 2 hours, and while the current is still on, the electrodes are removed and immediately washed with water. The washings are caught in the electrolytic beaker. The solution is neutralized with ammonium hydroxide, evaporated to a volume of 25 to 50 ml., and then transferred to a 100-ml. volumetric flask. Enough nitric acid is added to dissolve any R<sub>2</sub>O<sub>3</sub> precipitate which may have appeared after the addition of the ammonium hydroxide. An excess of the acid should be avoided. Manganese can be removed before or after evaporation by precipitation as the hydrous oxide in the solution obtained from the electrolysis.

After dilution to 100 ml., a 25-ml. aliquot is placed in a 100-ml. pear-shaped centrifuge tube. The solution is again brought back to the neutral point with ammonium hydroxide. Only a few drops of the hydroxide should be required. Seventy ml. of acetic acid are added, followed by 5 ml. of saturated ammonium oxalate solution. The procedure outlined for the titrimetric determination of zinc is then followed. The precipitates containing tin, lead, and copper are treated in the customary manner.

**CAST AND PHOSPHOR BRONZES.** Cast and phosphor bronzes are run in the same manner as the high-zinc alloys, except that the whole sample is used for the zinc determination instead of an aliquot. After electrodeposition of the copper the solution is evaporated in the electrolytic beaker to dense fumes of sulfur trioxide, diluted to 10 ml. with water, allowed to cool, neutralized with ammonium hydroxide, and transferred to a centrifuge tube. The beaker is rinsed with water until the volume of solution plus washings is 25 ml.; water rinsings are abandoned and glacial acetic acid is used for the final rinsings. Glacial acetic acid is added to the solution in the centrifuge tube until a volume of 9 ml. is reached. The regular titrimetric procedure is then followed.

The results obtained from following such a procedure appear in Table V. This procedure is fairly simple and can be carried out with fairly inexpensive equipment. Two or more samples can be centrifuged while other samples are being digested or titrated.

## ATTEMPT TO DETERMINE ZINC IN AN ALUMINUM ALLOY

Because of the favorable results obtained in the analysis of copper-base alloys, an attempt was made to determine zinc by



Table V. Determination of Zinc in National Bureau of Standards Samples

Sample	Zinc			Zinc Found		
	Present %	Found %	Difference %	Mean %	Av. deviation %	Av. deviation of mean %
Manganese bronze, B.S. No. 62	35.06	35.16	0.10	35.24	0.08	0.03
		35.20	0.14			
		35.25	0.19			
		35.47	0.41			
		35.25	0.19			
		35.12	0.06			
Zinc brass, N.B.S. No. 37b	27.09	27.27	0.18	27.07	0.07	0.03
		27.09	0.00			
		26.91	-0.18			
		27.03	-0.06			
		27.09	0.00			
		27.04	-0.05			
Copper bronze, N.B.S. No. 52	1.89	1.98	0.09	2.01	0.02	0.01
		2.00	0.11			
		2.02	0.13			
		2.02	0.13			
Phosphor bronze, B.S. No. 63	0.48	0.51	0.03	0.51	0.01	0.00
		0.52	0.04			
		0.52	0.04			
		0.50	0.02			
Zinc concentrate, B.S. No. 113	61.1	60.8	-0.3	60.9	0.2	0.1
		60.9	-0.2			
		61.2	0.1			
		61.2	0.1			
		60.9	-0.2			
		60.5	-0.6			

Calculated on basis of omission of one or two results whose deviation exceeds four times the average deviation.

Similar procedure in an aluminum base alloy, National Bureau of Standards Sample 86, containing 1.50% of zinc. The zinc oxalate could not be precipitated from the solution obtained by the dissolution of the alloy because the high concentration of zinc greatly interfered when the solution was adjusted for the precipitation of zinc oxalate. The addition of ammonia caused precipitation of aluminum hydroxide which could be filtered from the solution only if a very large volume were used. If this were done, a very large volume of filtrate was obtained. So much filtrate was consumed in the evaporation of such a large volume that the method was abandoned. A preliminary separation of zinc by hydrogen sulfide did not seem to present any advantages over the oxalate method, because it would be much easier to weigh the oxide which could be obtained by ignition of the sulfide precipitate.

#### DETERMINATION OF ZINC IN ZINC CONCENTRATES AND ORES

Two National Bureau of Standards samples, Nos. 113 and 2a, were analyzed for zinc content. Sample 113 was a zinc concentrate containing 61.1% of zinc. This sample has not been completely analyzed by the National Bureau of Standards; however, the following analysis in per cent has been reported for this concentrate: moisture, 0.05; acid-insoluble material, 4.97; calcium, 0.42; copper, 0.03; iron, 0.88; aluminum, not determined separately; lead, 0.33; calcium carbonate, 0.77; magnesium carbonate, 0.32; sulfur, 31.25; zinc (by difference), 60.99. Sample 2a was a synthetic one prepared from ores from all over the United States, containing a pure zinc blende from Joplin, Mo.; a mixture of franklinite, willemite, calcite, etc., from Franklin, N. J.; an impure blende from Colorado containing a good deal of iron, copper, and lead; and enough cadmium sulfide to give about 0.6% cadmium. The zinc content of this sample was 35.3% on the as-received basis.

**DETERMINATION OF ZINC IN ZINC CONCENTRATE.** A 0.20-gm sample is weighed into a porcelain casserole, 5 ml. of concentrated nitric acid are added, and the casserole is covered with a watch glass and placed on a steam bath. When only 3 ml. of solution remain, 7 ml. of concentrated hydrochloric acid are added and digestion is continued until only 2 or 3 ml. remain. Three to 4 ml. of concentrated sulfuric acid are added and the solution is evaporated well beyond the point of the appearance of the first sulfur trioxide fumes. The solution is cooled and diluted to 20 to 25 ml. The silica is then removed by filtering

the solution through No. 42 Whatman filter paper. The residue is washed several times with hot water. The filtrate which was collected in a 100-ml. volumetric flask is made just basic to modified methyl red and then made slightly acid with dilute sulfuric acid. The solution is finally cooled and diluted to 100 ml. Aliquot volumes of 25 ml. are analyzed for zinc by the titrimetric procedure described.

The results of analyzing the zinc concentrate appear in Table V. This method seems very applicable to rapid assay analysis of zinc concentrates of this type because the zinc can be determined immediately after the dissolution of the sample. The results obtained by this method agree within a few tenths of a per cent with that reported by the Bureau of Standards.

The attempts to analyze sample 2a failed to give very satisfactory results. This sample was of such a peculiar composition that it was believed that any procedure evolved would be useless for samples obtained from natural ores. A procedure for the analysis of this sample could probably be worked out, and would resemble that commonly used for limestone analysis with the added step of copper separation by electrolysis.

The procedure tried was as follows:

Dissolution was obtained by aqua regia. Silicon, iron, and calcium were removed according to methods recommended by the American Society for Testing Materials. The resulting solution was evaporated to dryness after the addition of 10 ml. of concentrated nitric acid and 20 ml. of concentrated hydrochloric acid. The residue was taken up with 10 ml. of dilute sulfuric acid (5 + 95) solution and the resulting solution evaporated to sulfur trioxide fumes. The residue was washed into a 100-ml. centrifuge tube with hot water. If 25 ml. of water were insufficient to obtain complete washing, glacial acetic acid was used to complete the washing. Enough acetic acid was added to make a 70% solution, and precipitation and titration of the oxalate were carried out as described.

The results varied from 29.2 to 31.2%, compared to 30.53% zinc reported by the National Bureau of Standards. Better results could probably have been obtained if copper had been separated before the precipitation of the zinc oxalate. A method such as this would be of value only if a complete analysis were carried out.

#### DISCUSSION

The method which has been developed seems to have several definite advantages in certain cases as compared to the phosphate and sulfide methods. When the diammonium phosphate method is applied in alloy analysis, it is often necessary to destroy the excessive amounts of ammonium salts prior to precipitation. Zinc oxalate can be precipitated in the presence of a fairly large amount of ammonium salts, which therefore need not be destroyed. The zinc ammonium phosphate is usually kept hot for 30 minutes and digested in the cold for at least 2 hours. The digestion of zinc oxalate rarely takes more than 1.5 hours. The end point of the permanganate titration is sharp even when very dilute standard solutions of potassium permanganate are used. The oxalate method appears to be more accurate for samples containing small percentages of zinc and probably would be more accurate for the larger percentages, if the entire sample were used instead of an aliquot. The use of the entire sample containing high percentages of zinc would, however, decrease the speed of analysis because of the time which would be consumed in evaporating the solution to 25 ml.

The separation of zinc sulfide is not an easy operation; therefore, when no interferences would occur, the oxalate separation would be preferred. In the analysis of ores and in certain types of alloys the sulfide precipitation appears to be preferable. In the complete analysis of a zinc ore, it might be advisable to determine zinc by the method proposed here. When only zinc is determined, the sulfide method of separation is more desirable. The application of the oxalate method to the determination of zinc in zinc concentrates seems to be very rapid for assay analysis. The accuracy of the determination will depend upon the interfering elements present. Procedures for analyzing concentrates



and ores should always be tested by a sample representative of the type to be analyzed.

The apparatus required for the oxalate method can be found in almost every analytical laboratory. Any type of centrifuge tube which allows the use of a large enough volume can be used. The centrifugal method of separation of precipitates is as rapid or more rapid than filtration, but washing by the centrifugal method is much easier and more complete. In many cases two washings are sufficient to remove all excess ammonium oxalate.

#### SUMMARY

Zinc can be completely precipitated as the oxalate in a 70% acetic acid medium. The zinc oxalate can be separated by use of a centrifuge or by filtration and dissolved in sulfuric acid. The oxalic acid formed can be titrated with a standard potassium permanganate solution. Ammonium nitrate and ammonium sulfate do not interfere with the precipitation, but ammonium chloride does. Elements which form oxalate precipitates in 70% acetic acid solution interfere.

A procedure was developed for the determination of zinc in brasses and bronzes. Four Bureau of Standards alloys were analyzed for zinc and favorable results were obtained. A procedure was attempted for aluminum-base alloys, but the high concentration of aluminum salts made the method unfeasible. An attempt was made to determine zinc in an ore but the sample which was available was of such an unusual composition that further work was inadvisable. A procedure for the determination of zinc in a zinc concentrate was developed and favorable results were obtained on a National Bureau of Standards sample.

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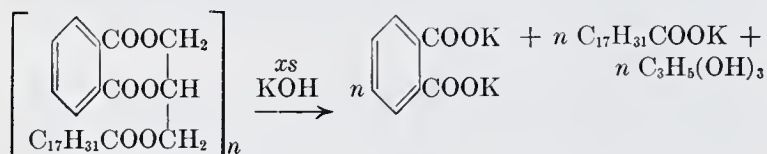
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# Determination of Total Phthalic Anhydride in Oil-Modified Alkyd Resins

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Several modifications of the Kappelmeier procedure for the determination of phthalic anhydride in oil-modified alkyd resins are proposed. Anhydrous potassium phthalate is recommended as the weighing form instead of the alcoholate. A simplified saponification procedure is suggested. The adverse effect of water and its elimination are described. A volumetric procedure is offered in place of the gravimetric one.

THIS determination of total phthalic anhydride, free and combined, in oil-modified alkyd resins has been the subject of a number of papers (3, 5, 8-11, 14, 15). In the case of the alkyd hypothetically represented below, dipotassium phthalate, potassium linoleate, and glycerol are obtained upon alkaline hydrolysis:



The monomer within the bracket is useful in representing the molecule for analytical purposes but should not be taken as an indication of the actual molecular structure.

The analytical problem involved is twofold: (1) to isolate the dibasic acid quantitatively and (2) to determine its quantity. Its determination as phthalic acid (3, 10), lead phthalate (5), potassium carbonate (14), potassium phthalate alcoholate (8), and

potassium sulfate (9) has been reported. Most of these methods are laborious, time-consuming, or subject to various errors (9, 10). The Kappelmeier method (8), which generally is accepted today, involves hydrolysis of the alkyd resin with alcoholic potassium hydroxide, precipitating potassium phthalate containing one molecule of alcohol of crystallization.

#### WEIGHING FORM

The necessity for drying the alcoholate to constant weight over concentrated sulfuric acid in a vacuum desiccator makes the weighing form inconvenient for routine work. Kappelmeier reported (8) that the alcohol is lost with difficulty at 100° C., that heating in air results in a brownish discoloration, and that drying the alcoholate in a vacuum between 100° and 120° C. results in a slow loss of weight until 16% has been lost, which corresponds to exactly one molecule of alcohol.

The tenacity with which the alcohol is held was verified. However, the alcoholate was decomposed in air quantitatively without discoloration by heating at 150° C. for 3 to 4 hours. The theoretical loss is 15.97%; 15.97 ± 0.07 was found (Table I). The treatment yields the anhydrous salt which is therefore recommended as the weighing form.

#### METHOD OF SAPONIFICATION

The standard method of saponification requires a preliminary heating below the boiling temperature, followed by a prolonged



Table I. Formation of Anhydrous Dipotassium Phthalate from the Alcoholate

Weight of $\text{C}_6\text{H}_4(\text{COOK})_2 \cdot \text{H}_2\text{O}$ Grams	Weight of $\text{C}_6\text{H}_4(\text{COOK})_2$ Grams	$\text{C}_2\text{H}_5\text{OH}$ Lost %	Weight of $\text{C}_6\text{H}_4(\text{COOK})_2 \cdot \text{C}_2\text{H}_5\text{OH}$ Grams	Weight of $\text{C}_6\text{H}_4(\text{COOK})_2$ Grams	$\text{C}_2\text{H}_5\text{OH}$ Lost %
676	1.409	15.90	1.634	1.374	15.92
824	1.532	16.01	1.879	1.579	15.97
462	1.226	16.14	2.110	1.772	16.02
162	0.977	15.92	1.512	1.272	15.87

Table II. Rate of Hydrolysis of Alkyd Resins

Temp. ° C.	Resin 1 Phthalic anhydride			Temp. ° C.	Resin 2 Phthalic anhydride		
	Hr.	%	Hydrolysis %		Hr.	%	Hydrolysis %
60	1	28.0	77.4	60	1	41.5	100
	2	36.0	100.0		2	42.0	100
	4	36.2	100.0		4	42.0	100
	8	36.0	100.0		8	42.0	100
50	1	19.3	53.4	50	1	42.0	100
	2	29.3	80.9		2	42.0	100
	4	35.3	97.5		4	42.0	100
	8	36.5	100.0		8	42.0	100
40	1	13.6	37.6	40	1	28.0	66.7
	2	22.0	60.9		2	42.0	100
	4	30.0	82.8		4	42.0	100
	8	35.3	97.5		8	42.0	100

gentle refluxing. This involves the use of water baths, air-cooled condensers, and the likelihood of violent bumping. A simplified method was investigated in view of the exceptionally rapid rate of hydrolysis of alkyd resins (16).

Similar-sized samples of a given resin were weighed into a series of corked Erlenmeyers. A solution of 0.5 *N* potassium hydroxide in absolute alcohol was prepared and equal volumes were added to each flask. They were then set in ovens maintained at the given temperature  $\pm 2^\circ \text{C}$ . for varying periods of time. At the end of this time they were placed in a water bath and cooled rapidly to room temperature, ether was added, and they were filtered into Gooch crucibles within 10 minutes after removal from the oven. The weight of phthalic anhydride was calculated on the resin solids basis, using the dipotassium phthalate alcoholate as the weighing form (15).

The extent of hydrolysis as a function of time may be calculated by considering the reaction complete at the point where maximum and constant value is obtained. The data in Table II indicated that refluxing is unnecessary. Indeed, saponification has been found to be complete in flasks which have been allowed to stand 18 hours at  $25^\circ \text{C}$ . It is recommended that saponification be carried out at  $55^\circ \text{C}$ . for 4 to 6 hours. It may be necessary to heat difficultly saponifiable resins overnight. Higher temperatures may result in building up excessive pressures within the flask and it is advisable to stopper the flask loosely.

This modification was tested upon two commercially prepared alkyd resins, using the customary reflux procedure as a control. Both the above weighing forms were used to secure the data in Table III. The modified saponification procedure gave results agreeing to  $\pm 0.35\%$ . This less drastic saponification procedure has been used successfully on hundreds of samples of oil-modified alkyd resins of the type of Rezyl 7818-1 (American Cyanamid Chemical Corp.), Beckosol 1334 (Reichhold Chemicals, Inc.), alkyd A-4-D (Falk and Co.), Glyptal ZV-1125 (General Electric Company), etc.

#### EFFECT OF WATER

The necessity of precipitating the dipotassium phthalate under anhydrous conditions has been noted (9). The quantitative effect of known quantities of water on the precipitation of dipotassium phthalate was observed.

The alcoholic potassium hydroxide was prepared by drying commercial absolute alcohol over lime for 48 hours, followed by

distillation over sodium. The fraction distilling over at  $78-79^\circ \text{C}$  was used to prepare the 0.5 *N* alcoholic potassium hydroxide. The c.p. potassium hydroxide contained 13% water and thus introduced 0.5% of water into the alcoholic potassium hydroxide solution. Solutions containing up to 8% more water were prepared by adding calculated quantities of water to this stock solution.

Table IV shows a maximum of 37.4% phthalic anhydride obtained where no water was added. An error of 1% is introduced in the presence of 2% added water. A further increase in water content leads to large errors. In another series of determinations where the same alkyd resin was analyzed using commercial absolute alcohol and c.p. potassium hydroxide the average of six determinations was  $37.05 \pm 0.05\%$ .

**PREPARATION OF LIMED ALCOHOLIC POTASSIUM HYDROXIDE.** For each determination 4.5 grams of c.p. potassium hydroxide are added to 125 ml. of absolute alcohol, 10 grams of calcium oxide, to remove a maximum of 2.5% of water, are added, and the solution is refluxed for 20 minutes. The warm solution is filtered rapidly with suction, a minimum of air being sucked through to avoid absorption of carbon dioxide.

To observe the effect of lime in the preparation of the alcoholic potassium hydroxide, two commercial resins were analyzed. Table V shows that the use of lime results in an increase of about 1% in the determination of phthalic anhydride. A comparison of columns A and C shows that the effect of the addition of ether is negligible. Apparently, only in the presence of an appreciable quantity of water does the ether decrease the solubility of the salt sufficiently to warrant its use (8).

#### VOLUMETRIC PROCEDURE

The advantages of the Kappelmeier procedure in isolating the phthalic anhydride as the dipotassium salt would be best realized if a convenient and accurate volumetric procedure could be developed. A volumetric procedure which partially fulfills these

Table III. Phthalic Anhydride by Kappelmeier Reflux and Modified Kappelmeier ( $55^\circ \text{C}$ .) Methods

	Resin 3		Resin 4	
	A %	B %	A %	B %
Reflux	35.67 35.53	35.69 35.56	36.43 36.42	36.45 36.42
$55^\circ \text{C}$ .	35.57 35.63	35.56 35.69	36.60 36.31	36.33 36.31

Weighing form. A =  $\text{C}_6\text{H}_4(\text{COOK})_2 \cdot \text{C}_2\text{H}_5\text{OH}$ . B =  $\text{C}_6\text{H}_4(\text{COOK})_2$ . Duplicate results listed.

Table IV. Effect of Water on Quantitative Precipitation of Dipotassium Phthalate

$\text{H}_2\text{O}$ Added %	Phthalic Anhydride %	Loss <sup>a</sup> %	$\text{H}_2\text{O}$ Added %	Phthalic Anhydride %	Loss <sup>a</sup> %
0.0	37.4	0.0	2.0	37.0	1.0
0.5	37.3	0.2	4.0	35.1	6.2
1.0	37.3	0.2	8.0	29.6	20.7

<sup>a</sup> Assuming 100% precipitation where no water was added.

Table V. Effect of Lime in Preparation of Alcoholic Potash in Modified Kappelmeier Procedure

Resin 3, Phthalic Anhydride			Resin 4, Phthalic Anhydride		
A %	B %	C %	A %	B %	C %
35.4	35.6	35.3	36.3	36.9	36.5
35.3	35.5	35.4	36.3	37.1	36.5
35.4	35.6	35.3	36.4	36.9	36.5
35.3	35.5	35.3	36.4	37.5 <sup>a</sup>	36.5

Results listed in quadruplicate

A Modified Kappelmeier, using normally prepared alcoholic potassium hydroxide.  
B Modified Kappelmeier, using "limed" alcoholic potassium hydroxide.  
C Same as A except for omission of ether before filtration of potassium phthalate.

<sup>a</sup> This sample contained only half the concentration of resin used in all the other samples.



Table VI. Titration of Dipotassium Phthalate with Perchloric Acid in Glacial Acetic Acid

Weight of $C_8H_4(COOK)_2$ Taken A	Calcd. Weight of $C_8H_4(COOK)_2$ from Titration B	Weight of $KClO_4$ Found C	Calcd. Weight of $C_8H_4(COOK)_2$ from $KClO_4$ D
Grams	Grams	Grams	Grams
0.928	0.935	1.061	0.929
0.986	0.995	1.131	0.990
1.154	1.162	1.329	1.160

requirements is based on the use of a feebly basic solvent to increase the basicity of the phthalate ion. Hall and Werner (7) have shown that perchloric acid is the most suitable of the common acids for titrimetric purposes in a glacial acetic acid solvent. The use of indicators in titrating amines with perchloric acid in glacial acetic has been reported (2).

The titration of dipotassium phthalate or its alcoholate can be followed with several indicators—for example, bromophenol blue, bromocresol purple, and methyl violet—the latter was very satisfactory (1, 4). It is necessary to remove the water present in the solutions to secure quantitative results (6). The addition of acetic anhydride converts the water to acetic acid; excess acetic anhydride has no effect on the titration (12). The anhydrous perchloric acid solution was standardized by the use of sodium carbonate as a primary standard (2), the end point being taken at the green-yellow stage. The perchloric acid presents a little danger in handling. The 0.1 N solution should be prepared carefully as indicated below. Once the perchloric acid is present in the dilute solution it is relatively harmless. The glacial acetic solutions are most conveniently used in a well-ventilated hood.

The standard solution of approximately 0.1 N perchloric acid in glacial acetic acid was prepared by adding 9 ml. of 60% perchloric acid dropwise to 36 ml. of acetic anhydride kept in a chilled glass-stoppered liter Erlenmeyer (7). The resultant solution was then diluted with 720 ml. of glacial acetic acid and 24 ml. of acetic anhydride and allowed to stand 2 weeks until the reaction with water was completed (13). To standardize the solution a definite quantity of sodium carbonate was dissolved with gentle warming in a solution of 20 ml. of glacial acetic acid and 2 ml. of acetic anhydride and brought to boiling (hood) in order to react with any water. Upon cooling, this solution was titrated with the above perchloric acid solution using methyl violet indicator, 2 drops per 10 cc. of 0.2% methyl violet in glacial acetic acid.

The results obtained by titrating dipotassium phthalate isolated by the modified Kappelmeier procedure are given in Table VI. A comparison of columns A and B shows that the volumetric procedure gives results that are 0.8% high. A white precipitate, formed upon addition of the perchloric acid, contained potassium and was soluble in hot water and insoluble in cold water. The precipitation of potassium perchlorate under these conditions has been noted (7). The precipitate was filtered off, dried to constant weight, and used as a basis for calculating the weight of dipotassium phthalate. A comparison of columns A and D indicates that precipitation is quantitative.

Sodium perchlorate is soluble in glacial acetic acid while the potassium salt is insoluble. A procedure for determining both potassium and sodium simultaneously under certain limited conditions is indicated. Semiquantitative results were secured on mixtures of potassium acetate and sodium carbonate.

ACKNOWLEDGMENT

The author wishes to express his gratitude to the Paint and Automotive Chemical Laboratory of the Army Ordnance Department now located in the Proving Center, Aberdeen Proving Ground, Md., where the laboratory work was performed.

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## Determination of Total Phthalic Anhydride in Modified Alkyd Resins

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THIS paper presents confirmatory evidence of the modified Kappelmeier method (3) described in the preceding paper (2), independently obtained during the spring of 1942 by a similar modification which is used in this laboratory. This modification, which also eliminates the tedious drying of the residue crystals over sulfuric acid, consists in removing the alcohol by crystallization by heating at 210° C. for one hour. The work done here indicates that the modification is applicable also to alkyd resins complicated by maleic anhydride or fumaric acid.

Table I. Alkyd Resin Analysis

Resin	Other Acids Present	Phthalic Anhydride		
		Standard method A %	Modified method B %	Modified method C %
1	Maleic anhydride	34.10	34.06	34.06
2	...	35.09	35.17	35.09
3	...	34.80	34.78	34.80
4	...	42.05	42.01	42.51
5	Maleic anhydride	35.42	35.43	35.41
6	...	34.78	34.84	34.61
7	Maleic anhydride	25.95	25.87	25.61
8	Maleic anhydride	17.94	17.87	17.87
9	Rosin	28.78	28.80	28.88
10	Fumaric acid	34.32	34.30	34.61
11	Fumaric acid	36.08	36.06	35.72

PROCEDURE. The standard Kappelmeier procedure (1) followed up to the point of drying the filtration residues over concentrated sulfuric acid in vacuum. The crucibles containing the residues are transferred from the low-temperature oven to 210° C. oven for one hour. They are then cooled over Dehydri-weighed, and converted to phthalic anhydride, assuming potassium phthalate as the weighing form.

Table I lists representative results on the basis of which the two methods may be compared.

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# Constant-Level Float Valve

WILBURN A. BOGGS, 2218-B. Cabrillo Ave., Torrance, Calif.

THE constant-level valve illustrated in Figure 1 makes it possible to fill a glass bottle with distilled water from the still reservoir without running it over and without giving it constant attention during the last stages of filling. It can be made from material available in any laboratory, and is efficient when gravity supplies the pressure. Its applications are numerous and varied. With the slit turned downward, the valve will release a vacuum drawing liquid into a bottle by by-passing the vacuum to the outside when the rising liquid opens the valve.

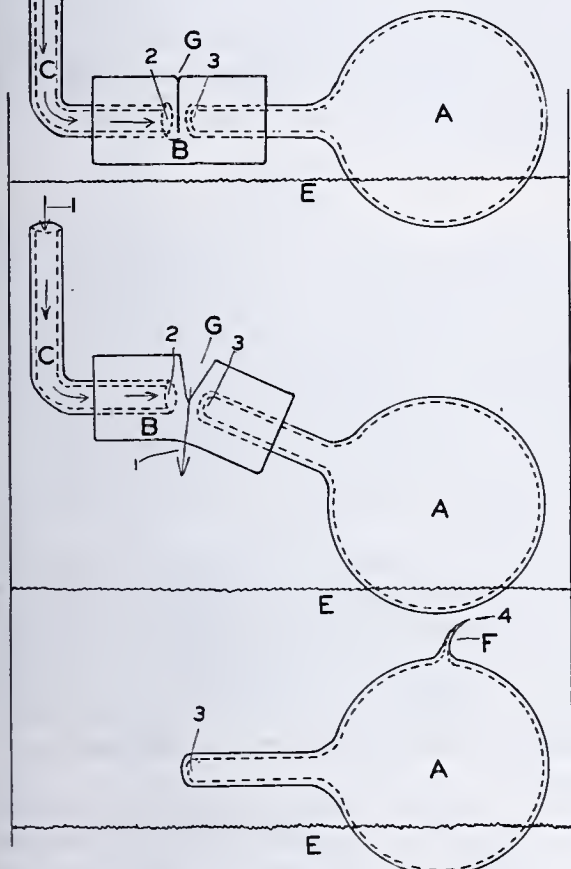


Figure 1. Float Valve

Upper. Valve closed.	Center. Valve open.	Lower. For use in hot water
A. Glass bulb	1. Liquid flow	
B. Rubber tubing	2. End open	
C. Glass tubing	3. End closed	
E. Liquid level	4. Fire-polished	
F. Capillary		
G. Slit		

VALVE. A piece of 7-mm. soft-glass tubing is heated in a flame and turned to thicken it at one end. When the glass is sufficiently thick, it is blown into a bulb 5 to 7.5 cm. (2 to 3 inches) in diameter. If it is to be used inside a bottle, the bulb could be as large as can easily pass through the mouth.

The length of the stem may be varied; the longer the stem the greater the closing pressure, but 2.5 to 3.75 cm. (1 to 1.5 inches) is a convenient length. The end is sealed off in the flame.

A piece of heavy-walled rubber tubing 3.75 cm. (1.5 inch) long is slit half through and the stem of the bulb is forced into the slit nearly up to the slit. A piece of 7-mm. glass tubing, bent at an angle, is forced nearly up to the slit from the other end. This glass tubing is held in position by running it through a cork stopper and holding the cork in a clamp. If the angle of the glass tubing is about 120° instead of 90° as shown, the closing effect is better and the rubber is kept above the liquid level.

The glass float must have a small hole in it, if it is to be used in hot water. This can best be made by heating the float on the inside, touching the heated portion with the molten end of a glass rod, and pulling out a fine capillary. When the end of the capillary is broken and fire-polished, a small hole is left, which will not fill with liquid. If sufficient heat is available, a small

round Pyrex flask may be fused to a piece of Pyrex tubing. This will usually withstand the pressure developed inside the bulb.

If the pressure is very great, the valve will squirt a small stream of water high into the air during the last stages of closing. This may be prevented by a guard:

A section of flat rubber (tube patching is good) is cut in a rectangular piece about as long as the rubber tubing of the valve and as wide as the circumference. Two strips of the same material are wrapped, one on the other, around the upper section of the rubber tube, and the rectangular piece is placed on top of them, extending well over the slit and down over the sides. These pieces should be wired in place, or fastened with a rubber band.

## Hot Distilled Water Reservoir

WILBURN A. BOGGS, 2218-B. Cabrillo Ave., Torrance, Calif.

THE arrangement shown in Figure 1, using the float valve described in the preceding article will provide a constant supply of about 6 liters (1.5 gallons) of hot distilled water per hour, if only a small amount (500 ml. or less) is drained off at a time. Fresh water is added continuously, but the volume is large enough to prevent variation of pH or temperature. The quality of the water is equal to that which has been boiled for 2 hours or longer, and is delivered from the buret at a temperature of above 98° C.

Tubes G, M, and N are placed in the positions shown and held in place by clamps above the bottle's mouth. The top of the bottle is left open to allow the steam to escape.

The steam coil is conveniently made by running 0.6-cm. (0.25-inch) copper tubing through a piece of pipe and then winding it around the outside of the pipe. The buret shown may be made from a Pyrex test tube (3.75 × 50 cm., 1.5 × 8 inches), to which a 7-mm. tube is attached. It is filled by opening pinchcock R<sub>1</sub> and emptied by R<sub>2</sub>.

The hole in the bottom of the Pyrex bottle is made by drilling with a piece of 1.9-cm. (0.75-inch) copper tubing rotated by hand or by a small motor. A small constant supply of wet Carborundum or valve-grinding compound must be supplied to the drill.

If steam is not available, the bottle is placed on a hot plate and supplemental heat furnished by a standard inside heating element. A more constant rate of heating can be attained by using a hot plate in conjunction with the steam coil.

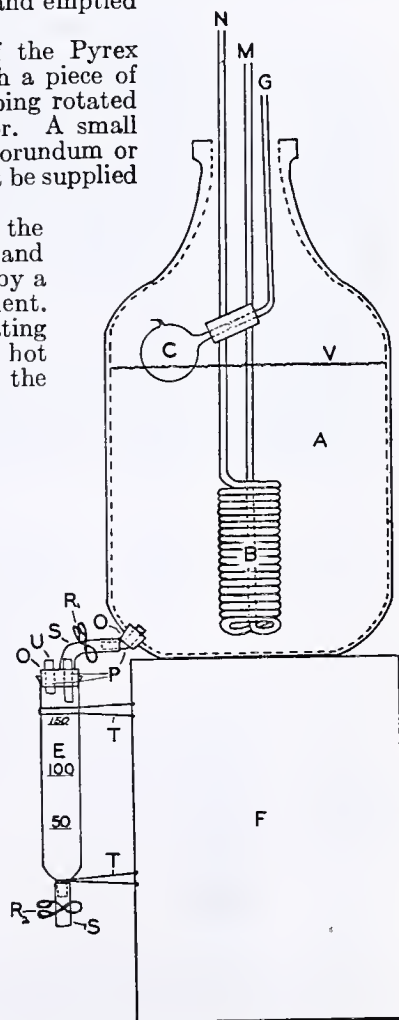


Figure 1. Reservoir

- A. Pyrex bottle, 5 gallons
- B. Closed steam coil
- C. Float valve
- E. Pyrex buret
- F. Base, 5-gallon can
- G. Distilled water inlet
- M. Steam inlet
- N. Steam outlet
- O. Rubber stoppers
- P. Glass tubing
- R. Pinchclamps
- S. Soft rubber tubing
- T. Stiff wire braces
- U. Buret exhaust
- V. Hot water level



# Mineral Contamination Resulting from Grinding Plant Samples

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Various grinding methods have been compared to determine the number of elements added during grinding and their relative seriousness as contaminants in preparation of plant samples. This paper reports the effects of type of method, type of plant material, duration of grinding, and size of plant sample upon contamination.

THE program of this laboratory is concerned with the nutritive value of food crops, and thus includes studies dealing with the importance of the micro nutrient elements in plant and animal nutrition. Several of these investigations require extensive mineral analyses of a variety of plant materials. Although a number of methods for grinding plant materials for analysis are available, a survey of the literature revealed few data concerning the mineral contamination resulting from different grinding methods.

Hamilton and Morris (3) considered it inadvisable to use iron mills in the preparation of feeds to be analyzed for iron. Two samples of unground corn were found to contain, respectively, 30 and 39 micrograms of iron per gram of sample, 56 and 67 micrograms after Wiley mill grinding, and 110 and 139 micrograms per gram after burr mill grinding. Nakamura and Mitchell (6) found that grinding corn in a Wiley mill increased the iron content from a value of 35 to 61 micrograms per gram, and that burr mill grinding increased the content to 125 micrograms per gram.

Preliminary investigations using common grinding methods revealed extensive contaminations and indicated the necessity of a more detailed study of both extent of and conditions affecting contamination.

### EQUIPMENT AND METHODS

The types of grinding equipment used were: Wiley mill, hammer mill, mortar and pestle, and jar mill, with flint, porcelain, and Mullite balls. (Mention of equipment and concerns by the U. S. Department of Agriculture does not constitute a recommendation or an endorsement. No discrimination is intended and no guarantee is implied.) The Wiley mill used was an A. H. Thomas Co., Size 1 mill, equipped with copper alloy screens. The hammer mill was manufactured by the Christy and Norris Co. of England. A No. 7 (23-cm.) Coors porcelain mortar and pestle was used to grind samples by hand. Two jar mills were used—a single-jar, 2-gallon, interior glaze, Abbe Engineering Co. porcelain mill, and an H. K. Porter Co., 12-place mill with 2-gallon, glazed porcelain jars manufactured by the Stevenson Co., Wellsville, Ohio. The porcelain balls were furnished with the 12-jar mill, the flint balls were supplied with the Abbe mill, and the Mullite balls were purchased from the Coors Porcelain Co.

Well-mixed samples of seeds were used in this investigation in order to obtain representative unground samples. Chemical analyses were carried out by the procedure of Parks, Hood, Hurwitz, and Ellis (7) for twelve biologically important elements: iron, zinc, copper, cobalt, molybdenum, manganese, phosphorus, sulfur, calcium, magnesium, potassium, and sodium. In addition, boron was determined by the method of Naftel. Duplicate analyses of single samples were made in all cases and the results reported on a dry weight basis. Of the total quantity of element in a given sample after grinding, the proportion which resulted from contamination due to grinding was designated as per cent contamination, using the increase over unground sample as the measure of contamination. By this method of calculation, a fourfold increase in mineral content is reported as 75% contamination, instead of the more common method of presenting it as a 300% increase. This avoids the cumbersome use of contaminations expressed as hundreds or thousands of per cent.

### RESULTS

Various grinding methods were compared to determine both the number of elements added during grinding and their relative seriousness as contaminants in plant sample preparation. In each comparison, the data for the elements which were not added as contaminants during grinding are omitted. In several of the tables, the content of a given element is slightly less in a ground sample than in the average of two unground samples. These differences are all within the limits of sampling and analytical error, as indicated by the analyses of duplicate unground samples (Tables I and II).

Table I shows contaminations introduced by various grinding methods. Iron and copper contaminations were high for all types of grinding except mortar and pestle. In addition, zinc, cobalt, and sodium were added in appreciable amounts by the jar mill. No significant contaminations of molybdenum, manganese, phosphorus, boron, sulfur, calcium, magnesium, or potassium were introduced by these grinding methods in this comparison.

Since the jar mill is extensively used in the preparation of samples for microelement analyses, a further comparison was made of contaminations introduced by various types of balls used in jar mill grinding. The comparisons in Table II for seven elements showing contamination by flint, porcelain, and Mullite balls indicate that the latter two materials are even more unsatisfactory than flint, both in the number of elements added and the relative magnitude of the contaminations. The remaining six elements determined were not added as contaminants.

The fact that jar mill balls wear appreciably during grinding is shown by Table III. In this comparison, the balls were washed, dried, counted, and weighed before and after grinding. The losses in weight of the balls during grinding indicate that appreciable wear took place. The amounts of rock flour recovered, compared to loss in weight of the balls, show that there was no significant wear on the mill

Table I. Composition of Oat Grain Samples

(Contamination resulting from various grinding methods)

Mill	Grinding	Iron		Zinc		Copper		Cobalt		Sodium	
		Con-	Con-	Con-	Con-	Con-	Con-	Con-	Con-	Con-	Con-
		tent	tamination <sup>a</sup>	tent	tamination <sup>a</sup>	tent	tamination <sup>a</sup>	tent	tamination <sup>a</sup>	tent	tamination <sup>a</sup>
		γ/g.	%	γ/g.	%	γ/g.	%	γ/g.	%	Mg/g.	%
None	Unground	24.9	...	21.9	...	4.0	...	0.01	...	0.25	...
None	Unground	...	...	21.3	...	3.5	...	0.01	...	0.32	...
Mortar and pestle	By hand	24.9	0.0	18.8	0.0	4.0	5.0	0.01	0.0	0.28	0.0
Wiley mill	10-mesh screen	38.2	35.0	21.0	0.0	9.0	57.8	0.01	0.0	0.32	9.4
Hammer mill	10-mesh screen	43.3	42.5	17.3	0.0	4.4	13.6	0.01	0.0	0.32	9.4
Jar mill, flint balls	19 hours	35.0	28.9	166.0	86.8	5.5	30.9	0.03	66.7	0.42	30.9

<sup>a</sup> Proportion of total quantity of element in a given ground sample, resulting from contamination due to grinding. Calculations based on averages of two unground samples.



Table II. Composition of Oat Grain Samples														
(Contamination resulting from different types of balls used in jar mill grinding)														
Type of Ball	Iron		Zinc		Copper		Cobalt		Sodium		Calcium		Sulfur	
	Con-tent	Con-tami-nation <sup>a</sup>	Con-tent	Con-tami-nation <sup>a</sup>	Con-tent	Con-tami-nation <sup>a</sup>	Con-tent	Con-tami-nation <sup>a</sup>	Con-tent	Con-tami-nation <sup>a</sup>	Con-tent	Con-tami-nation <sup>a</sup>	Con-tent	Con-tami-nation <sup>a</sup>
	γ/g.	%	γ/g.	%	γ/g.	%	γ/g.	%	Mg/g.	%	Mg/g.	%	Mg/g.	%
Unground sample	24.9	...	21.9	...	4.0	...	0.01	...	0.25	...	1.6	...	1.47	...
Unground sample	...	...	21.3	...	3.5	...	0.01	...	0.32	...	1.0	...	1.42	...
Flint <sup>b</sup>	35.0	28.9	166.0	86.6	5.5	30.9	0.03	66.7	0.42	30.9	1.2	0.0	1.40	0.0
Porcelain <sup>b</sup>	159.5	84.4	196.0	89.0	9.3	59.1	1.12	99.1	1.07	72.9	1.5	13.3	1.49	2.7
Mullite <sup>b</sup>	365.0	93.2	140.0	85.4	13.3	71.4	0.30	96.7	2.23	87.0	2.4	45.8	3.35	56.8

<sup>a</sup> Proportion of total quantity of element in a given ground sample, resulting from contamination due to grinding. Calculations based on averages of two unground samples.

<sup>b</sup> Grinding time, 19 hours.

Table III. Abrasion on Balls and Mill during Jar Mill Grinding <sup>a</sup>				
(Effect of different types of balls)				
Type of Ball	Initial Weight of Balls	Final Weight of Balls	Loss in Weight of Balls	Rock Flour Recovered <sup>b</sup>
	Grams	Grams	Grams	Grams
Porcelain	5361	5315	46	45
Mullite	6693	6644	49	72
Flint	4412	4397	15	16

<sup>a</sup> 11 days grinding, no plant sample. Probably 95-98% recovery.

when flint or porcelain balls were used, but a large amount wear on the walls of the mill jar with Mullite balls. This fact is notable, since the data indicate that the use of a tungsten carbide, or other balls which are harder than material of the jar will cause serious contamination due to wear on the jar wall.

To determine whether contamination is equally severe for different types of plant material, vetch seed and oat grain ground under identical conditions were compared. The striking differences in the amounts of contamination of zinc and copper (Table IV) were also found for iron, cobalt, and sodium.

Table IV. Composition of Oat Grain and Vetch Seed Samples									
(Effect of type of plant material on contamination resulting from various grinding methods)									
Mill	Grinding	Zinc		Vetch		Copper		Vetch	
		Con-	Con-	Con-	Con-	Con-	Con-	Con-	Con-
		tent	tami-	tent	tami-	tent	tami-	tent	tami-
		γ/g.	nation <sup>a</sup>	γ/g.	nation <sup>a</sup>	γ/g.	nation <sup>a</sup>	γ/g.	nation <sup>a</sup>
None	Unground	21.6	...	45.5	...	3.8	...	11.6	...
Wheat and rye	By hand	18.8	0.0	46.9	3.0	4.0	5.0	10.6	0.0
Wheat mill	10-mesh screen	21.0	0.0	42.0	0.0	9.0	57.8	11.7	0.9
Barley mill	10-mesh screen	17.3	0.0	45.7	0.4	4.4	13.6	11.1	0.0
Wheat mill, flint balls	19 hours	166.0	86.8	64.0	28.9	5.5	30.9	10.7	0.0

<sup>a</sup> Proportion of total quantity of element in a given ground sample, resulting from contamination due to grinding.

Loss in weight of balls during jar mill grinding with different types of plant material (Table V) substantiates the view that grinding contamination varies with type of plant material. Studies of the effect of duration of grinding on contamination of the elements are reported in Table VI. Although there is some evidence of an increase in contamination with increased time of grinding, the results are erratic for all types of mills. For the jar mill samples ground with Mullite balls, loss in weight of the balls during grinding was determined. When the contamination of the samples with iron is plotted against loss in weight of the Mullite balls during grinding (Figure 1), a direct correlation is found. A similar trend occurred for calcium, sodium, sulfur, zinc, cobalt, and copper. The unequal rates of wear of the balls

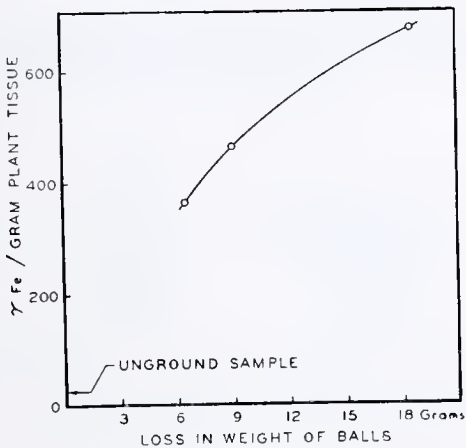


Figure 1. Iron Contamination of Oat Grain as a Function of Loss in Weight of Mullite Balls during Jar Mill Grinding

can probably be accounted for by nonuniform chipping, particularly with the flint pebbles, which exhibit a characteristic conchoidal chipping during grinding. The rock chips are never found as such in the plant samples.

The erratic nature of the contamination during Wiley mill grinding is less easily explained, but is readily verified. In Table VII are presented the iron analyses of six replicate oat grain samples, each ground once through the Wiley mill with uniform feeding from the hopper. The several-fold variation in contamination cannot be related to any of the factors being studied. In some laboratories a magnet is passed over such samples to remove loose particles of iron. The efficiency of this system might also be expected to vary with different types of plant material.

The contaminating elements in flint rock flour are present (Table VIII) in approximately the same proportion as in

contamination of the oat grain sample ground in the jar mill with flint pebbles. Because of the lack of homogeneity of the flint

Table V. Loss in Weight of Flint Balls during Jar Mill Grinding <sup>a</sup>			
(Effect of different types of plant material)			
Plant Sample	Initial Weight of Balls	Final Weight of Balls	Loss in Weight of Balls
	Grams	Grams	Grams
None	4412	4397	15
Clover <sup>b</sup>	4397	4386	11
Vetch <sup>c</sup>	4617	4614	3

<sup>a</sup> 11 days of grinding in each case.

<sup>b</sup> 100 grams of ladino clover. This sample completely filled the mill.

<sup>c</sup> 100 grams of vetch seed.



Table VI. Composition of Oat Grain Samples

Effect of time of grinding on contamination resulting from various grinding methods)

Mill	Grinding	Iron		Zinc		Copper	
		Con- tent γ/g.	Con- tami- nation <sup>a</sup> %	Con- tent γ/g.	Con- tami- nation <sup>a</sup> %	Con- tent γ/g.	Con- tami- nation <sup>a</sup> %
None	Unground	24.9	...	21.9	...	3.8	...
Wiley mill,	Once	38.3	35.0	21.0	0.0	9.0	57.8
10-mesh	Twice	61.6	59.5	20.0	0.0	9.9	61.6
screen	Four times	49.2	49.4	21.6	0.0	12.0	68.3
Jar mill,	19 hours	35.0	28.9	166.0	86.8	5.5	30.9
flint	33 hours	48.3	48.5	159.0	86.5	4.8	20.8
balls	66 hours	45.0	44.6	236.0	90.6	6.5	41.5
Jar mill,	19 hours	365.0	93.2	148.0	85.4	13.3	71.4
Mullite	33 hours	677.0	96.3	643.0	96.8	18.5	79.5
balls	66 hours	464.0	94.5	533.0	96.0	13.7	72.2

<sup>a</sup> Proportion of total quantity of element in a given ground sample, resulting from contamination due to grinding.

Table VII. Composition of Oat Grain Samples

(Erratic nature of contamination resulting from uniform Wiley mill grinding technique)

Replicate Samples	Grinding	Iron	
		Content γ/g.	Contamination <sup>a</sup> %
1	None	24.9	...
2	Mortar and pestle	24.9	0.0
3	Wiley mill <sup>b</sup>	65.0	61.7
4	Wiley mill <sup>b</sup>	194.0	87.1
5	Wiley mill <sup>b</sup>	72.5	65.7
6	Wiley mill <sup>b</sup>	219.0	88.7
7	Wiley mill <sup>b</sup>	136.0	81.7
8	Wiley mill <sup>b</sup>	158.0	84.4

<sup>a</sup> Proportion of total quantity of element in a given ground sample, resulting from contamination due to grinding.<sup>b</sup> 10-mesh screen, each sample ground once.

balls with respect to mineral content, successive grindings might be expected to introduce variable contamination of a given element.

Table IX illustrates the lack of homogeneity in the flint balls, by comparison of the analysis of a single pebble picked at random from the mill charge, with the analysis of the rock flour.

The effect of varying the size of plant sample used during jar mill grinding was also studied. The results for five of the elements showing contamination are illustrated in Figure 2. The increase in contamination with reduction in size of sample ground is very marked for these five elements. Calcium and phosphorus were also added in appreciable amounts during the grinding of the 30-gram sample.

## DISCUSSION

All the grinding methods studied except mortar and pestle gave serious contamination of one or more of the micro elements. As a result, the only satisfactory methods of plant sample preparation apparent at this time are use of mortar and pestle or not grinding the tissue. With care, it is possible to grind a plant sample by hand in a large mortar and pestle without losses and without contamination of any of the elements studied. Sampling becomes much more difficult when the tissue is unground. One partially satisfactory method of meeting this difficulty would be to take samples small enough to be ashed or digested in their entirety.

Other solutions of this grinding problem may be possible. Purr (8) found it possible to grind fresh animal tissue by freezing with liquid nitrogen and pounding with a hammer. To avoid the introduction of heavy metals, Kirk and Sumner (4) used a porcelain burr mill to grind jack beans in the preparation of urease. An attempt was made to use this mill in the present study. Although preliminary results indicated no contamination, the design is such that whole grains and stemmy materials cannot be satisfactorily ground. It seems likely that a similar porcelain burr mill could be designed which would be suitable for general plant tissue grinding. Chrome or cadmium plating of the inner surfaces of a hammer or Wiley mill might also be possible.

It is commonly recommended (1) that for iron or other micro element analyses, plant samples should be ground in a porcelain jar mill using flint pebbles, instead of in a Wiley or hammer mill. The basis of this recommendation is well known, since flint is of marine origin and contains appreciable amounts of carbonaceous material (2). The results obtained here, showing its mineral content and rate of wear during grinding, would not support this recommendation. It has been further recommended (1) that contamination arising during grinding in a Wiley or hammer mill may be compensated by subtracting a grinding blank. The results presented in Table VII do not substantiate this recommendation.

The grinding of plant samples for analysis for certain elements in a particular mill may be possible, but each mill and each type of plant material would have to be examined individually for each element to be determined.

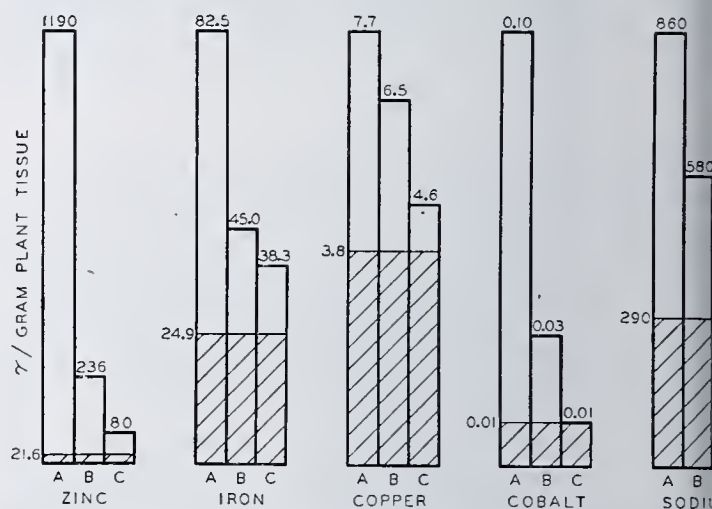


Figure 2. Contamination of Oat Grain Samples as a Function of Sample Size

30-, 120-, and 480-gram samples of oat grain (bars A, B, and C, respectively) were ground for 66 hours in a jar mill using flint balls. The 480-gram sample was not completely ground in this length of time. The content of each element is denoted by the heights of the bars. The shaded area represents the content of the element in the unground sample.

The data presented further indicate that many of the previous analyses and conclusions regarding micro element content of plants should be re-examined in light of the grinding procedure used.

## SUMMARY

In an investigation of contamination resulting from grinding plant tissues in the common grinding mills, the effects of type of grinding methods, type of plant material, duration of grinding, and size of plant sample upon contamination of thirteen elements during sample preparation were studied.

Grinding plant material in Wiley mill and hammer mill produced iron and copper contamination. Jar mill grinding with flint balls resulted in contamination of iron, zinc, copper,

Table VIII. Comparison of Composition of Flint Rock Flour with Contamination in a Plant Sample<sup>a</sup>

Element	Micrograms of Element per 0.03 Gram of Flint Rock Flour	Contamination of Plant Tissue, γ/g.
Zn	185.1	137.4
Na	141.0	130.0
Fe	13.7	23.4
Cu	0.8	1.0
Co	0.01	0.01

<sup>a</sup> Oat grain, ground 33 hours in jar mill, using flint pebbles.



Table IX. Comparison of Composition of Single Flint Ball and Flint Rock Flour Produced during Jar Mill Grinding

Element	Flint Flour Mg./g.	Single Pebble Mg./g.
Ca	11.7	...
Mg	0.06	0.06
K	6.6	1.5
Na	4.7	...
P	5.0	0.2
S	0.0	0.0
	$\gamma/g.$	$\gamma/g.$
Fe	458.	20.9
Zn	6170.	49.3
Cu	25.7	98.8
Mn	6.3	1.71
Co	0.35	0.4
Mo	10.0	

cobalt, and sodium. The use of porcelain or Mullite balls during jar mill grinding gave rise to appreciable contamination of iron, zinc, copper, cobalt, sodium, and in some cases calcium, sulfur, and phosphorus. Flint, porcelain, and Mullite balls wear appreciably during jar mill grinding. The contamination of elements in flint rock flour are present in about the same proportions as in the contamination of a plant sample ground in the jar mill, using flint balls.

Hand grinding with a mortar and pestle resulted in no appreciable contamination of iron, zinc, copper, boron, cobalt, manganese, molybdenum, calcium, sodium, magnesium, phosphorus, sulfur, or potassium.

Grinding contamination is erratic for both jar and iron mills. Several-fold variation in iron contamination resulted from uniform Wiley mill grinding technique. While iron contamination of samples ground in a jar mill using Mullite balls is almost a straight-line function of loss of weight of the balls during grinding, the loss in weight is not uniform with time.

Large increases in contamination of zinc, iron, copper, cobalt, and sodium resulted from decrease in size of plant samples ground in a jar mill using flint pebbles.

For the types of mills studied, less contamination resulted from the grinding of vetch seed than from the grinding of oat grain.

All the mechanical grinding methods used resulted in serious contaminations of one or more elements. Although a particular mill may be used in the preparation of plant samples for the analyses of certain elements, it is concluded that marked errors would be involved in using the common mills for grinding plant tissue for general or extensive micro element analyses.

#### ACKNOWLEDGMENTS

The Christy and Norris hammer mill and the porcelain burr mill were used through the courtesy of the Cornell University Departments of Agronomy and Biochemistry.

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## Glass Electrode Assembly for Titrating Microbiological Vitamin Assays

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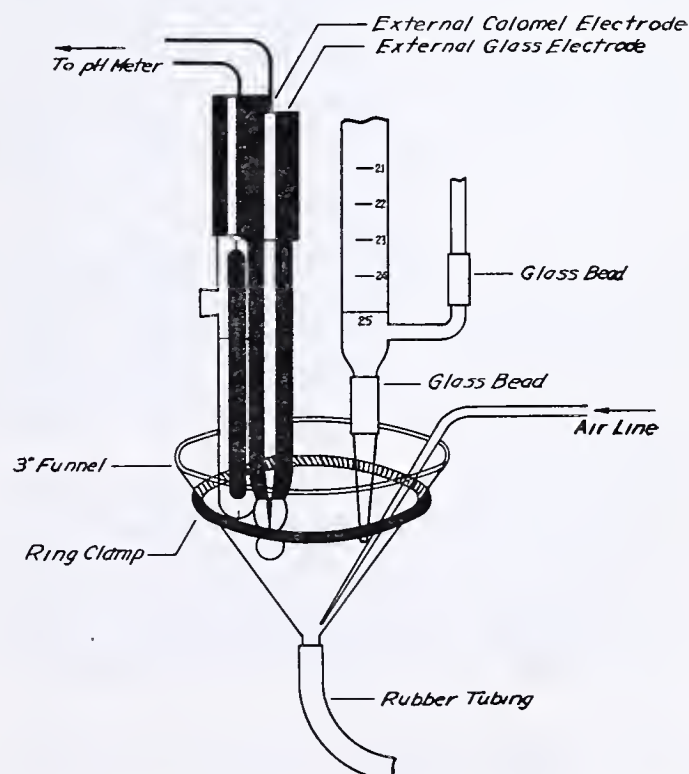
IN THE titration of microbiological vitamin assays it is often more desirable to use a glass electrode pH meter to determine the end point than to use an indicator and note the color change. A simple assembly, using a well-known commercial glass electrode pH meter (Beckman, Laboratory Model G) and standard laboratory equipment, has proved very satisfactory in this laboratory, furnishing a simple and rapid accurate titration procedure.

A 7.5-cm. (3-inch) glass funnel with the stem cut off 1.25 cm. (0.5 inch) from its body and fitted with rubber tubing long enough to reach to a drain is supported about 7.5 cm. (3 inches) from the laboratory bench. A small piece of glass tubing connected to the compressed air line through a trap is placed in the funnel, so that the bottom tip of the tube is just in the stem of the funnel. The top of a 25-ml. buret is inserted halfway down the funnel and over one side. An external glass electrode and calomel electrode are suspended in the funnel about 2.5 cm. (1 inch) from the bottom and slightly off center. A pinchclamp is inserted in the rubber tubing line just below the stem of the funnel.

To titrate an assay, the pinchclamp is put in place and the air turned on, so that a steady stream of bubbles will emerge from the air tube. The assay medium is poured into the funnel and the assay tube is washed with 10 to 12 ml. of distilled water, which is also added to the funnel content. The electrometric titration is carried out in the customary manner. The funnel is then drained by opening the pinchclamp, and the assembly is ready for the next titration.

#### ACKNOWLEDGMENT

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# CHLOROMETRY

## A Titrimetric Procedure Available for Microanalysis

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Standard sodium hypochlorite solution is recommended as a general titrimetric reagent for microanalytical work. The solution must be made with sufficient excess of sodium hydroxide so that the pH is about 12.5 and must be stored in brown, glass-stoppered bottles. Under these conditions, sodium hypochlorite solution is remarkably stable and compares favorably with other titrimetric reagents. With the use of this reagent, chlorometric determinations can be made at room temperature with ease, precision, accuracy, and a sharpness of the end point that leaves nothing to be desired in an analytical reagent.

IT IS a popular misconception that because sodium hypochlorite is a highly reactive substance it is too unstable to be used as a standard titrimetric reagent. Because of this belief, it has almost never been used for this purpose.

"Chlorometry" is the term used to designate the quantitative estimation of various substances by use of standard hypochlorite solution, in a manner entirely analogous to iodometry and bromometry. Although chlorometric determinations have not achieved any degree of practical application, there are references to the use of hypochlorite as a macroanalytical reagent in the literature.

As early as 1824, Gay-Lussac estimated the strength of chlorinated lime by permitting a fine suspension of chlorinated lime to flow from a buret into a hydrochloric acid solution of an arsenite and obtained the end point of the reaction by using indigo as the indicator. Denigès (3) using a similar modification turned to potassium bromide as the indicator. However, Lunge-Berl (9) showed both of these determinations to be rather inaccurate.

In some respects, chlorometry has as venerable a history as iodometry, introduced by Bunsen and Schwarz in 1853, the bromate methods of Koppeschaar (8) and the bromometric methods of Manchot (10). Jackson and Parsons (4) advocated the use of sodium chlorite as a volumetric oxidizing agent.

Jellinek and Kreteff (5), continuing a series of studies of newer methods in volumetric analysis, presented chlorometry as a valuable volumetric aid. They prepared a standard solution of sodium hypochlorite by passing chlorine gas from a small tank of chlorine into *N* sodium hydroxide solution until the solution was approximately 0.13 *N* with respect to sodium hypochlorite content. They kept this solution in a clear transparent bottle connected to a buret, using rubber connections, and found that with an excess of alkali the titer of the standard sodium hypochlorite solution kept surprisingly constant. Thus, their original titer, using the potassium iodide-hydrochloric acid method of estimation, was 0.1350 to 0.1351 *N*. After 7 days, the strength of this solution was practically unaltered, for their estimations gave the normality a value of 0.1351 to 0.1352. After an additional 10 days, the titer had been reduced to 0.1330 to 0.1328. They attributed this loss of about 1.5% to the fact that they had made no attempt to shield the solution from sunlight.

Jellinek and Kuhn (6) prepared a standard solution of sodium hypochlorite in the manner described by Jellinek and Kreteff and found it practically constant for a number of weeks.

Kolthoff and Stenger (7) found that "H.T.H." calcium hypochlorite yielded stable solutions. They added excess bromide to the sample to be titrated, so that in their titrations the added hypochlorite actually behaved as hypobromite. They standardized their hypochlorite solutions by titration against arsenic trioxide in acid or weakly alkaline solution, using Bordeaux as the indicator.

Chapin (2) in a study of the decomposition of hypohalites found that potassium hypochlorite solution had its maximum stability at pH 13.1.

### PREPARATION OF STANDARD SODIUM HYPOCHLORITE SOLUTION

Undoubtedly one reason for the lack of enthusiasm among analysts for the use of sodium hypochlorite as a titrimetric agent is the apparent difficulty of preparing such solution. The authors discarded the cumbersome method of Jellinek and Kreteff (5) and prepared a standard solution in the following simple manner.

Transfer 8.0 ml. of a commercial preparation of sodium hypochlorite solution containing 5% of available chlorine to a glass-stoppered brown-glass bottle, and dilute with water to about 2 liters. If necessary, add sufficient sodium hydroxide (1 gram) to raise the pH to about 12.5, the optimum pH for stability. To ascertain if the proper pH has been reached, the customary colorimetric methods for the determination of pH in the range 12 to 14 may be used. The authors used Chlorox as the source of sodium hypochlorite. Obtain the titer of the solution by titration against a primary standard of sodium arsenite made as follows:

Weigh 0.2473 gram of arsenious oxide (arsenic trioxide,  $As_2O_3$ , National Bureau of Standards) and dissolve in 25 ml. of 10% sodium hydroxide solution. Transfer to a 1-liter volumetric flask, make slightly acid with sulfuric acid (1 to 6), and dilute with water to 1 liter. This solution is 0.005 *N*.

The solution of sodium hypochlorite made as directed above is generally somewhat stronger than 0.005 *N*. Its exact titer can be determined by titration against the standard arsenite solution. Its normality may be adjusted to exactly 0.005 *N* by the usual procedure.

### TITRATION PROCEDURE

To estimate the strength of the sodium hypochlorite solution the following simple procedure may be used.

Transfer a known aliquot of standard arsenite solution to a 125-ml. Erlenmeyer flask or a 150-ml. beaker: a 4-ml. aliquot if a microburet is to be used for the standard hypochlorite solution and a 5-ml. aliquot if a semimicroburet is to be used. A standard solution of tartar emetic, [potassium antimony tartrate,  $K(SbO)C_4H_4O_6 \cdot \frac{1}{2}H_2O$ ], containing 1 mg. of antimony per 10 ml. of solution may also be used. Add 5 ml. of concentrated hydrochloric acid and adjust the volume of the solution to 35 to 40 ml. by adding distilled water. Fill a microburet or semimicro buret with the standard hypochlorite solution. Add 1 drop of 0.05% methyl orange indicator solution to the test solution and titrate directly with the sodium hypochlorite solution. Add another drop of methyl orange indicator solution near the end point and continue the titration until the color of the methyl orange is destroyed. Make a blank titration using exactly the same volume of hydrochloric acid, water, and drops of methyl orange indicator solution, replacing the volume of arsenite or antimony test solution by additional distilled water. The blank should run about 0.12 to 0.14 ml.

### RESULTS

In order to determine the stability of the standard sodium hypochlorite solution prepared in the manner directed above, titrations were performed as detailed at intervals during 3 years. In all, five series of experiments were run. Titrations were performed in triplicate and the results averaged. The results obtained in three of these series are representative. In the first series the initial normality was 0.004970; after 102 days it was still 0.004970; the maximum variation within this period was  $\pm 0.000008$ . In the second series, initial normality was 0.005570; after 175 days this had degraded to 0.005543, the maximum variation



within the period being  $-0.000021$ . In another series, the normality was  $0.00500$  and at the end of 56 days was  $0.00477$  with a maximum variation of  $-0.000020$ . The other series gave comparable results.

A check on the accuracy of sodium hypochlorite solution as a titrimetric reagent, arsenic and antimony were determined in standard solutions, using both the potassium bromate and the sodium hypochlorite methods.

#### DISCUSSION

In the determination of microquantities of antimony, the potassium bromate method (1) was not entirely satisfactory for a number of reasons, the principal ones being that titrations had to be performed almost at the boiling point in strong hydrochloric acid solution with the consequent production of very copious and irritating fumes of hydrogen chloride and that the blank, using methyl orange as the indicator, was large. The blank obtained using some of the indicators suggested by Smith and Bliss (11) was even larger.

A standard sodium hypochlorite solution has several marked advantages. It is an economical reagent. One can perform direct titrations with it. It is unnecessary to perform the titrations at elevated temperatures, eliminating any danger from the irritating fumes of hydrochloric acid. The blank is smaller than that obtained with potassium bromate titrations. Hypochlorite titrations can be performed under conditions of low acid concentration without apparent decrease in accuracy. From  $0.1$  to  $1$  mg. of antimony or arsenic per  $10$  ml. of sample solution can easily be determined. Titrations can be made in glass-stoppered bottles,

glass-stoppered Erlenmeyer flasks, or iodine flasks, if desired, to minimize losses attributed to volatility. Many of the indicators mentioned by Smith and Bliss, and Kolthoff and Stenger can be used instead of methyl orange without increase of the blank. The precision compares favorably with that of other methods, as can be seen from the reproducibility of results.

Several precautions must, however, be observed in using sodium hypochlorite solution as a titrimetric reagent. It must be preserved in brown, glass-stoppered bottles. It may be kept at room temperature without deterioration over considerable periods of time. Keeping the solution at lower temperatures is perhaps preferable.

The optimum conditions for the titrations are a volume of at least  $35$  to  $40$  ml. with an acid concentration equivalent to  $5$  ml. of concentrated hydrochloric acid.

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## Thiosulfate Washers in Alkoxy Microdeterminations

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Determinations of methoxy and methylimide groups in which thiosulfate alone is used as a washer give values considerably lower than theoretical. This is due to the solubility of the methyl iodide in the washer, and a subsequent reaction. Ethoxy and ethylimide determinations are not subject to this loss. The effect of thiosulfate can be eliminated by using as washer thiosulfate dissolved in saturated sodium chloride, or by adding cadmium sulfate as in the standard volumetric procedure. The minor errors in determinations using washers water, phosphorus suspension, or  $0.5\%$  sodium carbonate, are insignificant in comparison with that due to thiosulfate. A clear distinction between ethoxy and methoxy can be made by using a determination with a good washer, such as  $0.5\%$  carbonate, then with  $5\%$  thiosulfate; the methoxy value will be reduced to  $5$  to  $70\%$  of the original, while ethoxy remains unchanged.

WORK on alkaloids in this laboratory required the development of micromethods of alkoxy and alkylimide determination. The apparatus used was that of Pregl for alkoxy and that of Friedrich for alkoxy and alkylimide determinations, and the procedure was essentially that of modern textbooks of microchemistry. Three to  $5$  mg. of material were weighed on tinfoil, dissolved in phenol and acetic anhydride, heated with hydriodic acid, and passed through a washer of  $5\%$  thiosulfate containing  $0.5\%$  sodium carbonate. Final estimation was by the Viebock-Brecher method. Preliminary experiments with the Friedrich apparatus showed that the values obtained with vanillin and several alkaloids did not agree with theory, calculation being from first principles. The titration obtained with all methoxy-containing substances was only  $50$  to  $70\%$  of the theoretical, while ethoxy values agreed closely with theory. The method was then examined in detail and many

of the more obvious possible sources of error were eliminated. The same effect was found in the Pregl apparatus. The only factor not eliminated appeared to be the washing solution. Consequently, red phosphorus suspension, the original washer of Pregl (9), as well as water and  $0.5\%$  sodium carbonate was tried. These gave theoretical results in the Pregl apparatus, and values some  $5\%$  low in the Friedrich apparatus. The use of thiosulfate as a washer was then investigated, and the literature searched for counterindications to its use. Thiosulfate washers are almost universally used and recommended by the later workers in microchemistry.

#### EXPERIMENTAL

Known amounts of methyl iodide were introduced into the Friedrich apparatus without any hydriodic acid, drawn through various washers, and titrated in the ordinary way. With no washer, or with water, phosphorus suspension, or  $0.5\%$  carbonate the recovery was almost theoretical. With thiosulfate there was only  $50$  to  $65\%$  recovery, thus confirming the effect of thiosulfate.

A survey of results obtained with various washers in the Pregl apparatus is given for vanillin in Table I, and for phenacetin in Table II, which show that the effect of thiosulfate on the methoxy value is detected when  $1$  ml. of a  $1.5\%$  solution is used. This effect becomes much larger when  $5$  to  $10\%$  thiosulfate is used, while with very high concentrations ( $40\%$ ) theoretical values are again obtained. With ethoxy there was no detectable effect in any concentration.

#### DISCUSSION

The effect of thiosulfate is explained as a result of two factors: (1) the solubility of the alkyl halide in the washing solution, with



Table I. Methoxy Values on Vanillin (Theory 20.36)<sup>a</sup>

Washing Solutions	Volume of Washing Solution Ml.	Substance Mg.	Titration, 0.0188 N Thiosulfate Ml.	Methoxy %	Washing Solutions	Volume of Washing Solution Ml.	Substance Mg.	Titration, 0.0188 N Thiosulfate Ml.	Methoxy %
Water	0.5	3.790	7.44	19.1	5% thiosulfate	...	5.298	7.15	13.1
	...	3.210	6.66	20.1		...	4.022	5.97	14.4
	...	4.526	9.39	20.2					
	1.0	5.511	11.80	20.7	5% thiosulfate with 0.5% carbonate	1.0	5.885	8.51	14.1
	...	3.546	7.49	20.5	10% thiosulfate	...	3.568	5.24	14.3
0.5% sodium carbonate	0.5	4.292	8.52	19.3		0.5	4.531	6.08	13.1
	...	3.930	8.07	20.0		...	5.284	7.89	14.5
	...	3.552	7.56	20.7		1.0	4.172	6.61	15.4
	...	3.193	6.66	20.2		...	3.600	5.80	15.6
1.0% sodium bicarbonate	0.5	4.110	8.52	20.1	20% thiosulfate	1.0	3.692	6.05	15.9
	...	3.250	6.69	20.0		...	3.423	5.60	15.9
1.5% sodium thiosulfate	0.5	3.881	7.57	19.0	40% thiosulfate	1.0	3.767	8.08	20.2
	...	4.117	8.34	19.7		...	3.892	8.30	20.7
	...	3.350	6.69	19.7	80% thiosulfate	1.0	5.252	10.39	19.2
	...	3.492	7.29	20.3	5% thiosulfate in saturated sodium chloride	1.0	5.470	10.80	19.2
	1.0	5.454	10.11	18.0		...	3.780	7.87	20.2
	...	3.217	6.61	20.0		...	4.900	10.29	20.4
	...	3.112	5.60	17.8		...	4.959	10.32	20.2
	...	3.388	6.53	18.7		...	3.008	6.21	20.1
	...	3.770	6.00	15.5					
5% thiosulfate	0.5	5.710	9.50	16.2	2.5% thiosulfate and 2.5% cadmium sulfate	1.0	5.090	10.45	20.0
	...	4.038	7.68	18.5		...	4.420	9.11	20.0
	...	4.026	7.49	18.0		...	3.545	7.43	20.4
	1.0	3.668	5.80	15.4		...	3.944	8.33	20.8
						...	5.049	10.71	20.6

Table II. Ethoxy Values on Phenacetin (Theory 25.14)<sup>a</sup>

Washing Solutions	Volume of Washing Solution Ml.	Substance Mg.	Titration, 0.0188 N Thiosulfate Ml.	Ethoxy %
Water	0.5	4.744	8.75	26.0
	...	5.334	9.62	25.4
	...	2.787	4.94	25.1
5% thiosulfate	...	4.213	7.57	25.3
	1.0	4.051	7.47	25.8
	...	5.680	9.91	24.6
	0.5	3.666	6.59	25.3
10% thiosulfate	1.0	4.815	8.65	25.3
	...	4.117	7.50	25.7
20% thiosulfate	1.0	5.211	9.25	24.9

<sup>a</sup> Several blanks gave 0.05 to 0.07 ml. of thiosulfate with the washers used, though a few with high thiosulfate concentrations gave up to 0.20 ml. When bromine was removed by several drops of formic acid, the resulting solution gave no detectable liberation of iodine from potassium iodide. The phenol method of Nanji (8) was not needed.

a consequent dependence on the volume of washer used, and (2) the rate of reaction of the dissolved halide with thiosulfate. Methyl iodide and thiosulfate react in water according to a well-known bimolecular reaction investigated by Slaton (11). At 25° C. his data show for 0.035 N thiosulfate and 0.018 N methyl iodide a half-time of 10 to 12 minutes, with  $K_2 = 0.85$ . The ethyl iodide reaction is slower with  $K_2$  at 25° = 0.050. This reaction is expected to occur in methoxy determinations because of the appreciable solubility of methyl iodide in water. At 20° 1.40 grams of methyl iodide dissolve in 100 grams of water (7). With very concentrated thiosulfate the solubility is apparently depressed, and little if any reaction can take place. The solubility of methyl iodide in concentrated neutral salt solutions would be expected to be much less than in water. With 1 ml. of 5% thiosulfate in saturated salt, no loss was found. Addition of an equal volume of 5% cadmium sulfate also completely suppressed any reaction, apparently through more than a solubility effect. In the case of ethyl iodide the lower rate of reaction and the lower solubility (at 20° 0.401 gram of ethyl iodide dissolves in 100 grams of water, 7) combine to give no detectable effect.

Satisfactory results were obtained by Pregl (9), using phosphorus suspension as a washer for alkoxy and alkimide determinations by Viebock and Brecher (13), and by many later workers using modified apparatus. The main purpose of the washer appears to be to remove hydrogen iodide vapors, rather than iodine itself, and for this purpose an aqueous washer appears effective even in alkimide determinations. Phosphorus suspension was criticized by Friedrich (6) as it is incapable of removing iodine rapidly. He therefore used for the gravimetric method 3% thiosulfate to which was added an equal volume of

5% cadmium sulfate. The use of the latter material for removal of hydrogen sulfide was due to Edlbacher (3). Friedrich's values were correct, as would be expected from this study, but he does not insist on the admixture with cadmium sulfate. Friedrich (5) states that use of cadmium sulfate is not necessary if the acid is free from sulfide. The standard washer for the gravimetric method (Roth, 10) is 1 ml. of equal volumes of 5% thiosulfate and cadmium sulfate. As indicated in the table this washer is perfectly satisfactory, and because of a chance effect which is not quite expected, the thiosulfate effect is eliminated, and results are free from error. This washer has also been used in modified volumetric methods by Elek (4) Christensen, Friedman, and Sato (1), and Cooke and Hibbert (2) with good results.

Slotta and Haberland (12) used for the volumetric method 1 ml. of 1.5% thiosulfate to which was added 0.5% sodium carbonate, and gave a few examples which were close to theory. From Table I it is seen that with 1 ml. of thiosulfate of this concentration a lowering is detected, but with 0.5 ml. little if any lowering. The washer for the volumetric method generally adopted by Friedrich (5) and by Roth (10) is 1 ml. of 5% thiosulfate with optional addition of 0.5% carbonate. Roth states that this washer was used by Slotta and Haberland. The carbonate was found to have no effect on the thiosulfate reaction and this washer was incapable of giving anything near the theoretical results. Friedrich (5) states that he used this washer for 5 years with completely satisfactory results. He gives no example of calculation and makes no mention of empirical corrections. In the volumetric methylimide determination he states that 1 ml. of 0.01 N thiosulfate = 0.1502 mg. of  $\text{CH}_3(\text{N})$ , which divided by 6, is in agreement with theory. Roth (10) also makes no mention of low results or empirical corrections. He gives 1 ml. of 0.02 N thiosulfate = 0.6204 mg. of  $\text{OCH}_3$ , which divided by 6 is theoretical. His factors given for calculation of methoxy, ethoxy, methylimide, and ethylimide are all in agreement with theory, assuming 100% recovery of alkyl iodide.

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## Chemical Analysis by Powder Diffraction

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This paper cites examples of use of the powder diffraction method in the chemical identification of solids, and discusses some of the general difficulties that may be encountered in its use.

THE past ten years the powder diffraction method has been used to an increasing extent in the chemical identification of solids (4, 5, 9, 12, 13, 16, 24, 26, 30, 34, 46, 47, 59, 60, 62). The utility of the method resides in its ability to detect the state of chemical combination for each crystalline component in a mixture (2). The present paper cites a few typical examples and discusses some of the general difficulties that may be encountered in this physical method of chemical analysis.

Identification of boiler deposits is a general problem that is conveniently handled by the powder method. A particular scale submitted for identification was examined by chemical analysis, by microscopic examination, and by powder diffraction. Table I lists the data ascertained by each method.

The information obtained by the diffraction method is of evident value to the chemist interested in selecting the correct treatment for the removal of scale or in devising methods to diminish scaling. The manner in which the diffraction data are conveniently compared with published powder data is illustrated in Table II.

Other typical problems solved by the diffraction method are: the identification of corrosion products, the constitution of fluxes, the detection of pigments or fillers in plastics or elastomers, the analysis of minerals, the recognition of allotropic modifications, and the study of chemical reactions in the solid state.

As illustrated in Table II, chemical analysis by the powder method consists of matching the diffraction pattern of an unknown material with one or more standard powder patterns. This requires (1) a correct registration and measurement of the diffraction lines and (2) a careful interpretation and evaluation of these data as applied to chemical analysis. The first part of the problem has received considerable attention in the literature and only a few points require enumeration. The second part, however, has not been treated extensively in previous publications and deserves detailed discussion.

COMPLICATIONS IN REGISTRATION OF POWDER  
DIFFRACTION PATTERNS

ABSORPTION EDGES. In view of the fact that most x-ray diffraction work is being done with filtered radiation, characteristic

Table I. Data on Boiler Scale

Chemical Analysis	Microscopic Analysis	Powder Diffraction Analysis
%		%
Fe 28.32	Black shiny powder,	~40 FeFe <sub>2</sub> O <sub>4</sub>
Ca 22.30	heterogeneous; particles too small for refractive index measurements	~30 CaCO <sub>3</sub> , calcite
S 6.21		~30 CaSO <sub>4</sub>
CO <sub>2</sub> 22.10		
Ignition loss 15.70		
	Advantages	
Accurate quantitative data, method of analysis independent of state of aggregation of sample	High sensitivity for detecting minor phases, morphological details readily ascertainable (29)	Direct identification of compounds, nondestructive method applicable to extremely fine powders

Table II. Powder Diffraction Data on Boiler Scale

Filtered MoK $\alpha$  radiation used to obtain powder diffraction patterns.  $d$  = interplanar spacing measured in Å.  $I$  = peak intensity of a diffraction line as estimated with a direct comparison intensity scale (arbitrary units).  $I/I_1$  = relative intensity of a diffraction line, where  $I_1$  is intensity of strongest line of particular phase in question. Most representative value for  $I_1$  of phase 1 is taken as 33 and is obtained by multiplying intensities of six unambiguous reflections (4.83, 2.95, 2.41, 1.705, 0.967, 0.855 Å.) by corresponding relative intensities of FeFe<sub>2</sub>O<sub>4</sub> and taking arithmetic mean of computed values of  $I_1$  (36, 55, 37, 22, 25, 20). The average value for the strongest line of phase 2 is 22; for phase 3 it is 38.

Spectroscopic analysis of scale: Fe, Ca, chief constituents; Co, Mo, Cu, Na, 0.001 to 0.01%.

Boiler Scale	FeFe <sub>2</sub> O <sub>4</sub>	Phase 1	CaSO <sub>4</sub>	Phase 2	CaCO <sub>3</sub>	Phase 3	$\Sigma I$
$d$ , Å. $I$	$d$ , Å. $I/I_1$	$I/I_1$	$d$ , Å. $I/I_1$	$I/I_1$	$d$ , Å. $I/I_1$	$I/I_1$	
5.80 1	.. ..	.. ..	.. ..	.. ..	.. ..	.. ..	..
4.83 3	4.85 0.06	0.09	.. ..	.. ..	.. ..	.. ..	..
3.87 4	.. ..	.. ..	3.89 0.03	(1)	3.86 0.08	(3)	4
3.49 25	.. ..	.. ..	3.49 1.00	1.14	.. ..	.. ..	..
3.03 30	.. ..	.. ..	.. ..	.. ..	3.04 1.00	0.79	..
2.95 10	2.97 0.28	0.30	.. ..	.. ..	.. ..	.. ..	..
2.84 15	.. ..	.. ..	2.85 0.67	0.68	.. ..	.. ..	..
2.52 75	2.53 1.00	(33)	.. ..	.. ..	2.49 0.20	(8)	41
2.41 6	2.42 0.11	0.18	.. ..	.. ..	.. ..	.. ..	..
2.33 8	.. ..	.. ..	2.32 0.33	0.36	.. ..	.. ..	..
2.27 10	.. ..	.. ..	2.26 0.01	(0.2)	2.28 0.24	0.26	..
2.21 8	.. ..	.. ..	2.20 0.33	0.36	.. ..	.. ..	..
2.08 30	2.10 0.32	(11)	2.08 0.11	(2)	2.09 0.20	(8)	21
1.99 1	.. ..	.. ..	1.99 0.11	(2)	.. ..	.. ..	..
1.91 10	.. ..	.. ..	1.93 0.04	(1)	1.92 0.32	0.24	..
1.865 12.5	.. ..	.. ..	1.86 0.27	(6)	1.87 0.24	(9)	15
1.745 4	.. ..	.. ..	1.74 0.20	0.18	.. ..	.. ..	..
1.705 6	1.71 0.16	0.18	.. ..	.. ..	.. ..	.. ..	..
1.640 4	.. ..	.. ..	1.64 0.27	0.18	.. ..	.. ..	..
1.606 20	1.61 0.64	(21)	1.59 0.03	(1)	1.60 0.16	(6)	28
1.560 1	.. ..	.. ..	1.56 0.05	0.05	.. ..	.. ..	..
1.518 6	.. ..	.. ..	1.52 0.07	(2)	1.51 0.12	(5)	7
1.480 30	1.483 0.80	(26)	1.487 0.08	(2)	1.475 0.05	(2)	30
1.439 4	.. ..	.. ..	.. ..	.. ..	1.439 0.08	0.11	..
1.420 1	.. ..	.. ..	1.420 0.08	(2)	1.425 0.05	(2)	4
1.320 2	1.326 0.06	(2)	1.318 0.09	(2)	1.350 0.03	(1)	5
1.278 6	1.279 0.20	(7)	1.275 0.09	(2)	.. ..	.. ..	9
1.210 2	1.210 0.05	(2)	1.215 0.05	(1)	.. ..	.. ..	3
1.150 2	.. ..	.. ..	.. ..	.. ..	1.150 0.05	0.05	..
1.120 2	1.121 0.10	0.06	.. ..	.. ..	.. ..	.. ..	..
1.090 7	1.092 0.32	0.21	.. ..	.. ..	.. ..	.. ..	..
1.047 6	1.049 0.10	(3)	.. ..	.. ..	1.045 0.06	(2)	5
1.010 2	.. ..	.. ..	.. ..	.. ..	1.011 0.03	0.05	..
0.967 4	0.970 0.16	0.12	.. ..	.. ..	.. ..	.. ..	..
0.938 2	0.940 0.06	0.06	.. ..	.. ..	.. ..	.. ..	..
0.879 2	0.880 0.10	0.06	.. ..	.. ..	.. ..	.. ..	..
0.855 4	0.859 0.20	0.12	.. ..	.. ..	.. ..	.. ..	..
0.850 1	0.853 0.08	0.03	.. ..	.. ..	.. ..	.. ..	..
0.810 1	0.814 0.10	0.03	.. ..	.. ..	.. ..	.. ..	..



Table III. Preparation of Powder Specimens

Method of Preparing Powder Specimen	Precautions to Be Observed				
	Influence of atmosphere (H <sub>2</sub> O, O <sub>2</sub> , CO <sub>2</sub> , dust.)	Abrasion contamination	Mechanical strain	Volatilization	Phase decomposition
Grinding or filing	Grind and load sample in a drying box flushed with purified dry N <sub>2</sub> ; or grind sample under dry benzene and load wet powder into a thin-walled glass capillary (31, 63)	Grind hard nonferrous materials in an agate mortar. (Micromonizer may be useful in the pulverization of abrasives.) Remove metallic iron contamination from steel mortar by magnetic methods	Vacuum-anneal metallic filings at suitable temperature (31)	Grind sample containing sublimable substances in a small closed system or in an equilibrium - atmosphere [NH <sub>4</sub> HCO <sub>3</sub> , (NH <sub>4</sub> ) <sub>2</sub> CO <sub>3</sub> , NH <sub>4</sub> OCO-NH <sub>2</sub> , NH <sub>4</sub> Na, NH <sub>4</sub> CN, NH <sub>4</sub> SH, etc.; ammonates or alcoholates may lose NH <sub>3</sub> or ROH on grinding]	(Supersaturated solutions, quenched alloys, pressure-sensitive substances such as wurtzite or brookite chemical reaction in solid state (14, 68). For alloys a structure integrating camera micro design may be adaptable)
Precipitation or crystallization	Coprecipitation or crystallization. Make qualitative spectroscopic analysis and spot tests on washed precipitate	Oxidation. Load precipitate in wet condition—e.g., pyrophoric catalysts such as Raney nickel	Solvation. Vacuum-dry washed precipitate		
Condensation, electrodeposition, or sputtering	Preferred orientation. Check condensed film for preferred orientation of crystallites by taking exposures at various inclinations	Oxidation (Adsorbed O <sub>2</sub> , H <sub>2</sub> O, etc., on substrate)			

Table IV. Powder Diffraction Pattern of Magnesium Oxide

(hkl)	d <sub>obs.</sub>	d <sub>calcd.</sub>	(I/I <sub>1</sub> ) <sub>obs.</sub>	(I/I <sub>1</sub> ) <sub>calcd.</sub>
111	2.425	2.428	0.08	0.08
200	2.103	2.103	1.00 (50)	1.00
220	1.487	1.487	0.50 (25)	0.55
311	1.268	1.268	0.04	0.06
222	(1.216)	1.214	0.12 (6B)	0.15
400	(1.053)	1.052	0.04 B	0.06
331	(0.9661)	0.9649	0.01 B	0.03
420 α <sub>1</sub>	0.9405	0.9405	....	..
420 α <sub>2</sub>	0.9404	0.9405	....	..
422 α <sub>1</sub>	0.8583	0.8585	....	..
422 α <sub>2</sub>	0.8587	0.8585	....	..

Type B1:  $a = 4.206 \pm 0.001 \text{ \AA}$ . ( $25^\circ \pm 3^\circ \text{ C.}$ )

EXPOSURE. Filtered MoK $\alpha$  radiation; camera radius, 20.477  $\pm$  0.010 cm., calibrated with spectroscopically pure Mg ( $a = 3.2026 \text{ \AA}$ ,  $c = 5.1998 \text{ \AA}$  at  $25^\circ \text{ C.}$ ); slit dimensions,  $0.2 \times 5 \times 50 \text{ mm.}$ ; specimen radius, 0.2 mm.; duplex film without intensifying screen.

PREPARATION OF MgO. Sublimed magnesium metal, spectroscopically pure, was dissolved in  $\approx 10\%$  HCl (c.p. grade distilled). Magnesium hydroxide was precipitated by dropwise addition of freshly prepared c.p. ammonium hydroxide. The precipitate was washed twice by decantation, followed by filtration and washing. The wet hydroxide was transferred to a clean ignited sillimanite crucible and vacuum-dried over Mg(ClO<sub>4</sub>)<sub>2</sub>. The Mg(OH)<sub>2</sub> was then converted to MgO by heating to  $890^\circ \text{ C.}$  for 1 hour in an oxygen atmosphere free of carbon dioxide.

CHEMICAL ANALYSIS. % Mg =  $60.22 \pm 0.05$  (pyrophosphate method); calcd. % Mg = 60.32.

SPECTROSCOPIC ANALYSIS FOR IMPURITIES. Si 0.01 to 0.1%, Al 0.005 to 0.05%, B 0.001 to 0.005%, Cu 0.001 to 0.005%.

PARTICLE SIZE DISTRIBUTION. Electron microscope, 200 to 1200  $\text{\AA}$ ; average diameter 650  $\text{\AA}$ , very uniform; particles are not of regular shape, though crystalline in character; general tendency is for platelike particles  $\sim 200 \text{ \AA}$ . thick (see Figure 1).

x-ray absorption manifests itself in four ways: (1) in the silver bromide emulsion ( $\text{AgK}\infty = 0.4845 \text{ \AA}$ ,  $\text{BrK}\infty = 0.9181 \text{ \AA}$ ); (2) in the filter (17) ( $\text{ZrK}\infty = 0.6874 \text{ \AA}$ ,  $\text{NiK}\infty = 1.484 \text{ \AA}$ ); (3) in the specimen containing elements, the characteristic absorption edges of which fall within the transmitted background radiation; and (4) in the tungsten contamination of the target ( $\text{WL}_1 = 1.022 \text{ \AA}$ ) (2). There also exists the possibility that the zinc edge ( $1.281 \text{ \AA}$ ) may introduce a discontinuity in the response of fluorazure intensifying screens. For powder diffraction work these effects are observed only for the more intense reflections. For example, using a sealed-off Mo tube operated at 35 kv., one finds that the intense (111) reflection of cubic silver iodide produces one spectrum toward the longer wave-length end of  $\text{AgK}\infty$  and another corresponding to  $\text{IK}\infty$ . In the case of silver phosphate the intense portion of the continuous spectrum from (210) is slightly stronger than the (220) MoK $\alpha$  reflection. For powder patterns the sharpness of the edge of the transmitted spectrum is often blurred; consequently care must be exercised in distinguishing these edges from weak K $\alpha$  reflections.

FLUORESCENCE. Fluorescence excited in the sample by primary K $\alpha$  radiation is readily detected by a general enhancement of the background and a corresponding decline in the prominence of the desired diffraction pattern. Using MoK $\alpha$  radiation for specimens containing yttrium, strontium, rubidium, bromine, or selenium, one obtains relatively weak diffraction patterns with high background. For example, carbon tetrabromide gives an

extremely poor powder pattern with MoK $\alpha$  but a normal intensity pattern with CuK $\alpha$ . Specimens containing elements of high atomic number—e.g., gold, mercury, thallium, lead, bismuth—show L-fluorescence with MoK. These fluorescence considerations apply not only to filtered K $\alpha$  radiation but also to crystal-monochromatized x-rays. In the case of filtered x-rays, the distribution of the continuous radiation may noticeably affect fluorescence. Thus if a zirconium oxide screen is placed in front of the film to filter the diffracted MoK radiation, the resultant diffraction pattern from a molybdenum specimen has a background 3 times as high as in the case when the filter is placed in front of the slit system. For general routine analysis by powder diffraction two sources of K $\alpha$  radiation—e.g., MoK $\alpha$  and CuK $\alpha$ —are usually adequate to avoid serious cases of fluorescence.

RESOLUTION. The geometrical factors deciding the resolution of a Debye-Scherrer-Hull pattern are:  $R$ , radius of the camera;  $r$ , radius of the powder specimen;  $h$ , length of the specimen;  $\delta$ , divergence of the impinging x-ray beam; and  $\sigma$ , difference in wave length of the K $\alpha$  doublet. For a fixed camera radius the optimum resolution corresponds to the limiting condition:  $r$ ,  $\delta$ ,  $\sigma \rightarrow 0$ . In general, however, the hardness of the primary radiation used and the time required for the registration of a good diffraction pattern are the limiting factors in the design of a suitable camera.

The geometrical resolution for a camera of radius  $R$  can be expressed approximately as the sum of  $\delta$ , the divergence of the diffracted beam due to the divergence or convergence of the impinging primary beam, and  $\omega_{r,h}$ , the angular spread of the diffracted beam due to the dimensions of the powder specimen

( $\omega_{r,0} = \frac{2r}{R}$  radian, for parallel x-rays) (8, 32, 41). Accordingly two powder reflections  $d$  and  $(d + \Delta d)$  will be resolved if

$$(\Theta\alpha_2 - \Theta\alpha_1)d < (\Theta_d - \Theta_d + \Delta d)\alpha > (\delta + \omega_{r,h})\theta$$

where  $\Theta$  is the Bragg angle for the interplanar spacing,  $d$ , and denotes the wave length under consideration (33). For example with MoK $\alpha_1$  radiation the two powder reflections corresponding to 2.260  $\text{\AA}$ . and 2.274  $\text{\AA}$ . will be separated by 3.4', which interval is equal to the MoK $\alpha$  doublet separation,  $(\Theta\alpha_2 - \Theta\alpha_1)_{2.26}$ . Evidently the use of very narrow slits does not aid greatly in the resolution of a noncubic powder pattern because of the disturbing doublet separation. (The utility and precision of powder diffraction work would be greatly enhanced if an intense source of strictly monochromatic  $\text{WL}\alpha_1$  could be made available for precision-focusing cameras). Convenient standards for testing and calibrating powder cameras are 200-mesh powders of the following substances: pure fused magnesium oxide, pure crystalline silicon, annealed 99.9% molybdenum metal, c.p. thallous chloride, and indium sesquioxide prepared by the direct oxidation of 99.95% indium metal.

ABSORPTION. The intensity distribution of a powder diffraction line is markedly influenced by absorption in the powder



Table V. Ambiguities Arising from Solid Solution or Isomorphism

Diffraction patterns taken with filtered MoK $\alpha$  radiation.  $d$  = interplanar spacing;  $I/I_1$  = relative intensity

Substance	$d$ , Å.	2.85	2.47	1.74	1.490	1.428	1.134	1.105										
	$I/I_1$	1.00	0.50	0.50	0.50	0.17	0.17	0.17										
b.15Na	$d$ , Å.	2.85	2.47	1.74	1.487	1.424	1.131	1.102										
	$I/I_1$	1.00	0.37	0.20	0.31	0.07	0.07	0.07										
Examples: In, In-Ag; Mg, Mg-In; Pd, Pd-Ag; Fe, Fe-Cr																		
	$d$ , Å.	2.36	2.04	1.445	1.232	1.179	1.022											
	$I/I_1$	1.00	0.53	0.27	0.53	0.05	0.01											
	$d$ , Å.	2.35	2.03	1.440	1.227	1.173	1.019											
	$I/I_1$	1.00	0.53	0.33	0.40	0.09	0.03											
Examples: Co, Ni; Cr, Fe; Pd, Pt; Rh, Ir; Mo, W; Cb, Ta																		
	$d$ , Å.	3.12	2.70	1.91	1.63	1.353	1.240	1.104										
	$I/I_1$	1.00	0.08	0.60	0.30	0.06	0.08	0.06										
	$d$ , Å.	3.12	2.69	1.91	1.63	1.353	1.242	1.104										
	$I/I_1$	1.00	0.05	0.75	0.50	0.05	0.18	0.15										
Examples: NaBr, PbS; NaCl, Ag(Cl, Br); KCN, RbCl; Mg <sub>2</sub> Sn, Mg <sub>2</sub> Pb; FeF <sub>2</sub> O <sub>4</sub> , NiMn <sub>2</sub> O <sub>4</sub> , ZnFe <sub>2</sub> O <sub>4</sub> ; Er <sub>2</sub> O <sub>3</sub> , Y <sub>2</sub> O <sub>3</sub>																		
	$d$ , Å.	3.33	2.60	2.35	2.27	2.10	1.75	1.67	1.56	1.492	1.412							
	$I/I_1$	1.00	0.80	0.06	0.20	0.05	0.80	0.20	0.08	0.11	0.32							
	$d$ , Å.	3.37	2.64	..	2.30	..	1.75	1.67	1.58	1.496	1.420							
	$I/I_1$	1.00	0.86	..	0.29	..	0.86	0.29	0.14	0.14	0.14							
	$d$ , Å.	3.34	2.64	2.36	2.30	2.11	1.75	1.67	1.58	1.492	1.435							
	$I/I_1$	1.00	0.63	0.18	0.03	0.02	0.63	0.10	0.05	0.10	0.10							
(H <sub>4</sub> ) <sub>2</sub> CrO <sub>4</sub>	$d$ , Å.	5.6	4.80	3.91	3.74	3.65	3.45	3.18	2.94	2.84	2.77	2.32	2.01	1.87				
	$I/I_1$	0.62	1.00	0.35	0.10	0.10	0.40	0.75	0.40	0.20	0.10	0.62	0.15	0.05				
(H <sub>4</sub> ) <sub>2</sub> SeO <sub>4</sub>	$d$ , Å.	5.5	4.80	3.89	..	..	3.45	3.17	2.95	..	2.75	2.33	1.98	1.87				
	$I/I_1$	0.60	1.00	0.40	..	..	0.40	0.80	0.40	..	0.20	0.40	0.20	0.10				
(Cl <sub>2</sub> .6H <sub>2</sub> O)	$d$ , Å.	5.6	4.85	3.52	3.11	2.94	2.73	2.56	2.40	2.20	2.07	2.02	1.98	1.89	1.86	1.81		
	$I/I_1$	1.00	1.00	0.31	0.10	0.63	0.50	0.20	0.63	0.50	0.05	0.03	0.31	0.20	0.10	0.03		
(Cl <sub>2</sub> .6H <sub>2</sub> O)	$d$ , Å.	5.5	4.85	3.53	3.08	2.95	2.70	2.54	2.40	2.18	2.05	2.02	1.97	1.90	1.86	1.81		
	$I/I_1$	1.00	1.00	0.50	0.04	0.50	0.50	0.30	0.50	0.50	0.06	0.06	0.20	0.20	0.14	0.02		
Examples: CoSO <sub>4</sub> .H <sub>2</sub> O(?), ZnSO <sub>4</sub> .H <sub>2</sub> O, NiSO <sub>4</sub> .7H <sub>2</sub> O, ZnSO <sub>4</sub> .7H <sub>2</sub> O Co(NH <sub>4</sub> ) <sub>2</sub> (SO <sub>4</sub> ) <sub>2</sub> .6H <sub>2</sub> O, Fe(NH <sub>4</sub> ) <sub>2</sub> (SO <sub>4</sub> ) <sub>2</sub> .6H <sub>2</sub> O, Zn(NH <sub>4</sub> ) <sub>2</sub> (SO <sub>4</sub> ) <sub>2</sub> .6H <sub>2</sub> O, Cu(NH <sub>4</sub> ) <sub>2</sub> (SO <sub>4</sub> ) <sub>2</sub> .6H <sub>2</sub> O																		

Table VI. Partial Ambiguities Due to Isomorphism

$d$  = interplanar spacing;  $I/I_1$  = relative intensity. In the case of mixtures the above differences are more difficult to check

Substance	$d$ , Å.	4.80	3.80	3.40	3.06	2.52	2.40	2.34	2.19	2.10	2.05	1.97	1.91
ZnSO <sub>4</sub> .H <sub>2</sub> O	$I/I_1$	0.64	0.11	1.00	0.40	0.40	0.06	0.11	0.14	0.10	0.05	0.13	0.08
MgSO <sub>4</sub> .H <sub>2</sub> O	$d$ , Å.	4.82	..	3.38	3.07	2.55	2.40	2.33	2.19	2.10	2.05	1.97	1.90
	$I/I_1$	0.40	..	1.00	0.20	0.40	0.05	0.03	0.05	0.04	0.17	0.05	0.05
MgSO <sub>4</sub> .7H <sub>2</sub> O	$d$ , Å.	5.9	5.3	4.50	4.22	3.76	3.41	2.96	2.87	2.74	2.66	2.48	2.38
	$I/I_1$	0.20	0.20	0.08	1.00	0.10	0.12	0.18	0.20	0.08	0.40	0.02	0.05
ZnSO <sub>4</sub> .7H <sub>2</sub> O	$d$ , Å.	..	5.3	4.50	4.20	3.76	3.44	2.99	2.87	2.75	2.66	2.50	2.37
	$I/I_1$	..	0.60	0.16	1.00	0.20	0.30	0.12	0.30	0.10	0.25	0.12	0.08
CoCo <sub>2</sub> O <sub>4</sub>	$d$ , Å.	4.68	2.86	2.43	2.34	2.02	..	..	1.65	1.56	..	1.432	1.235
	$I/I_1$	0.08	0.20	1.00	0.06	0.13	..	..	0.04	0.25	..	0.30	0.02
ZnAl <sub>2</sub> O <sub>4</sub>	$d$ , Å.	..	2.85	2.44	..	2.02	1.91	1.85	1.65	1.55	1.480	1.431	1.232
	$I/I_1$	..	0.53	1.00	..	0.07	0.07	0.07	0.13	0.33	0.07	0.40	0.07
Mn <sub>3</sub> Al <sub>2</sub> (SiO <sub>4</sub> ) <sub>3</sub>	$d$ , Å.	2.89	2.59	2.46	2.36	2.27	2.10	2.04	1.88	1.67	1.60	1.545	1.44
	$I/I_1$	0.30	1.00	0.02	0.20	0.13	0.15	0.05	0.25	0.15	0.30	0.40	0.085
Mg <sub>3</sub> Al <sub>2</sub> (SiO <sub>4</sub> ) <sub>3</sub>	$d$ , Å.	2.88	2.58	2.45	2.35	2.26	2.10	2.03	1.87	1.66	1.595	1.54	1.440
	$I/I_1$	0.50	1.00	0.18	0.20	0.20	0.15	0.02	0.25	0.13	0.40	0.63	0.13
(NH <sub>4</sub> )Al(SO <sub>4</sub> ) <sub>2</sub> .12H <sub>2</sub> O	$d$ , Å.	7.0	5.4	4.97	4.30	4.07	3.67	3.26	3.05	2.95	2.79	2.60	..
	$I/I_1$	0.30	0.60	0.30	0.80	0.60	0.40	1.00	0.30	0.08	0.12	0.12	..
KAl(SO <sub>4</sub> ) <sub>2</sub> .12H <sub>2</sub> O	$d$ , Å.	7.0	5.4	4.96	4.29	4.05	3.65	3.24	3.03	2.93	2.78	2.58	..
	$I/I_1$	0.04	0.20	0.08	1.00	0.40	0.04	0.40	0.16	0.12	0.20	0.06	..
KCr(SO <sub>4</sub> ) <sub>2</sub> .12H <sub>2</sub> O	$d$ , Å.	7.0	5.5	4.98	4.31	4.08	3.68	3.26	3.04	..	2.81	2.59	..
	$I/I_1$	0.12	0.16	0.08	1.00	0.30	0.60	0.35	0.30	..	0.12	0.06	..
K <sub>2</sub> CaFe(CN) <sub>6</sub>	$d$ , Å.	6.0	5.1	4.60	3.72	3.64	3.07	2.85	2.57	2.35	2.30	2.13	2.06
	$I/I_1$	0.07	0.30	0.03	0.13	1.00	0.10	0.03	0.42	0.03	0.10	0.07	0.10
K <sub>2</sub> CuF <sub>2</sub> (CN) <sub>6</sub>	$d$ , Å.	..	5.1	..	..	3.63	3.06	2.86	2.57	2.36	2.29	2.13	2.06
	$I/I_1$	..	0.38	..	..	1.00	0.20	0.08	0.75	0.15	0.31	0.25	0.63
As <sub>2</sub> O <sub>3</sub>	$d$ , Å.	6.3	3.18	2.75	2.53	2.24	2.12	1.95	1.66	1.59	1.54	1.438	..
	$I/I_1$	0.56	1.00	0.24	0.32	0.08	0.16	0.24	0.16	0.08	0.16	0.08	..
Sb <sub>2</sub> As <sub>2</sub> O <sub>6</sub>	$d$ , Å.	6.4	3.18	2.75	2.52	2.24	2.11	1.95	1.66	1.58	1.54	1.430	..
	$I/I_1$	0.25	1.00	0.25	0.13	0.02	0.04	0.30	0.30	0.06	0.10	0.07	..



subdivision, such as coprecipitated oxides of the spinel type, specially prepared catalysts, or clays give rather diffuse diffraction patterns that may appear insignificant in the presence of an intense sharply defined pattern. A similar situation is encountered in the field of high polymers. In order to obtain information of greater value in the case of these idiosyncracies, the use of crystal-monochromatized radiation has been advocated by a number of diffractionists.

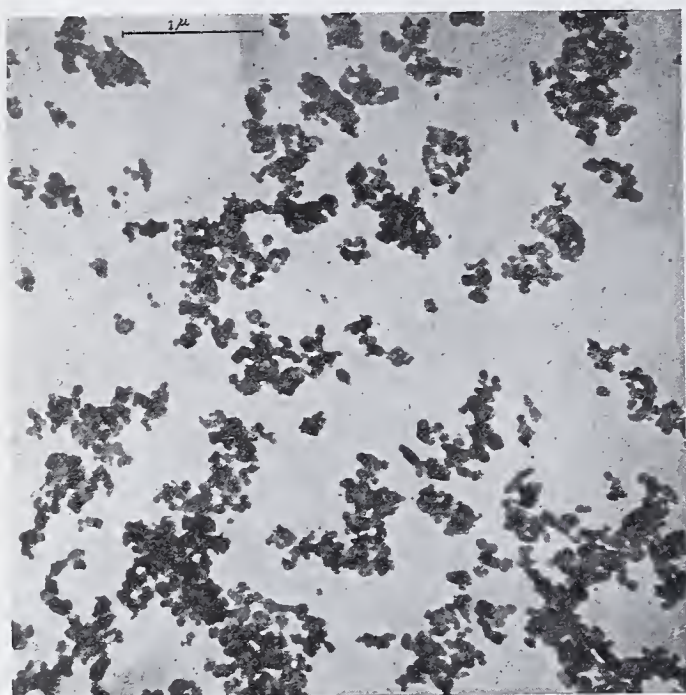


Figure 1. Electron Micrograph of Magnesium Oxide Standard

The problem of the instability of a sample usually can be overcome without serious difficulty (see Table III). Hygroscopic substances are readily powdered in a drying box and loaded into thin-walled glass capillaries that can be sealed easily. Efflorescent materials can be loaded wet (to prevent extreme crystal growth, a little amorphous material, either starch or charcoal, is admixed with the wet powder). In the case of complex organic compounds the existence of two or more allotropic modifications must be kept in mind; thus a material crystallized from a melt may give an entirely different powder pattern than the same substance crystallized from a solution. Other cases of less general occurrence are: allotropic transformation on grinding or filing (wurtzite, brookite, supersaturated solid solutions); photosensitivity ( $\text{BaN}_6$ ,  $\text{P}_4$ ); and chemical interaction of the substance with glass capillaries [acid fluorides, such as  $\text{NH}_4\text{FHF}$  to form  $(\text{NH}_4)_2\text{SiF}_6$ ]. Polystyrene capillaries, developed for the study of acid fluorides, have proved useful for powder diffraction with soft radiation such as  $\text{CuK}\alpha$  and  $\text{FeK}\alpha$ .

#### COMPLICATIONS IN METHOD OF ANALYSIS

Chemical analysis by the Debye-Scherrer-Hull method consists of matching the diffraction pattern of an unknown material with one or more standard powder patterns. When a match is found (see Table II) one infers that compounds  $A, B, C, \dots$  are present in the unknown. If  $p$  denotes the powder diffraction data of the unknown material;  $p_r$ , the diffraction data of all the cataloged standard patterns; and  $\Omega'$ , the composite operation of matching the unknown with the standards  $A, B, C, \dots$  one may express the mode of analysis symbolically as

$$\Omega'(p.p_r) \supset A, B, C \dots$$

The uniqueness of this inference is not evident and requires further examination.

**VALIDITY OF STANDARD PATTERNS.** It is convenient to consider first the validity of the standard patterns,  $p_r$  (4, 25, 26). From the results of x-ray diffraction analysis it is well recognized that the powder diffraction pattern of a single phase is a function of the particular crystal structure, the elements present, and the crystallite-size distribution of the powder. To be acceptable as a standard each substance should be subjected to a precise analysis to substantiate the assigned chemical formula. The method of preparation of the powder specimen should also be stated. Table IV illustrates a satisfactory presentation of a powder diffraction standard.

On examining the published tabulated powder data (4, 25, 26) one finds the labeled standards usually have not been examined for the correctness of the ascribed chemical formulas. This deficiency was realized when the first 1000 Dow standards were published (25).

The more frequent types of errors are: (1) incorrect degree of hydration [pattern 240 is  $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ ; pattern 338,  $\text{CoSO}_4 \cdot \text{H}_2\text{O}$ ; pattern 623,  $\text{NiF}_2 \cdot 4\text{H}_2\text{O}$ ; pattern 864,  $\text{Na}_2\text{S} \cdot x\text{H}_2\text{O}$ ]; (2) chemical reaction between standard substance and water, oxygen, or carbon dioxide [pattern 193 is mixture of  $\text{CaCO}_3$ ,  $\text{Ca}(\text{OH})_2$ , and graphite (?); pattern 227,  $\text{Ca}_{10}(\text{OH})_2(\text{PO}_4)_6$  and  $\text{Ca}(\text{OH})_2$ ; pattern 215, vaterite plus calcite]; and (3) incorrect chemical formula [pattern 13 is  $\text{Al}(\text{OH})_3$ , gibbsite; patterns 68 and 69 are largely  $(\text{Sb, As})_4\text{O}_6$ ; patterns 132 and 143 are largely  $\text{Bi}(\text{OH})_3$ ; pattern 269 refers to a mixture containing minor amount of hexamethylene tetramine]. Some errors in the diffraction data have been noted—e.g., the most intense line for  $\text{FeCl}_2 \cdot 2\text{H}_2\text{O}$  (pattern 409) is 5.5 Å. (40); the (020) reflection for  $\text{Na}_2\text{SO}_4 \cdot \text{H}_2\text{O}$  (pattern 859) should read 4.47 Å.

An examination of the useful compilation of powder data on minerals (26) reveals a similar state of affairs for these tabulated standards:

Pattern 3 (alabandite,  $\text{MnS}$ ) lists in addition to the diffraction data for cubic manganese sulfide the lines 2.83 Å. and 1.995 Å. (probably  $\text{NaCl}$ ). Pattern 23 [bravoite,  $(\text{Fe, Ni})\text{S}_2$ ] gives the accepted C2 pattern plus the noncubic reflections 3.08, 2.18, 2.01, 1.86, and 1.682 Å. Pattern 35 (chromite,  $\text{FeO} \cdot \text{Cr}_2\text{O}_3$ ) does not correspond to the  $\text{Hl}_1$  structure for  $\text{FeCr}_2\text{O}_4$ . Pattern 37 (clausthalite,  $\text{PbSe}$ ) lists an extraneous 2.86 Å. reflection. In pattern 39 (coloradoite,  $\text{HgTe}$ ) the strong (111) reflection is missing and the lines 2.82 Å. and 2.52 Å. appear extraneous, as does the line 2.79 Å. in pattern 95 (magnetite,  $\text{Fe}_3\text{O}_4$ ). Pattern 93 (löllingite,  $\text{FeAs}_2$ ) does not check the published data (53); the specimen used may have been an impure form of arsenopyrite. Pattern 1 (sphalerite,  $\text{ZnS}$ ) checks the B3 structure except line 3.95 Å. Pattern 143 (sylvanite,  $\text{Cu}_3\text{VS}_4$ ) omits the (220) reflection (1.9 Å.) and lists the extraneous lines 5.2, 4.15, 3.70, and 2.84 Å.

For a number of substances the innermost reflections have not been recorded: pattern 9 (argentite,  $\text{Ag}_2\text{S}$ ) should include the line 3.91 Å. (57); pattern 16 (berthierite,  $\text{FeS} \cdot \text{Sb}_2\text{S}_3$ ), 8.0, 7.5, 5.7, 5.1 Å.; pattern 66 (gersdorffite,  $\text{NiAsS}$ ), 3.99 Å.; pattern 77 (hausmannite,  $\text{Mn}_3\text{O}_4$ ), 3.52 Å.; pattern 77 (hausmannite,  $\text{Mn}_3\text{O}_4$ ), 4.92 Å.; pattern 95 (magnetite,  $\text{Fe}_3\text{O}_4$ ), 4.85 Å.; pattern 1 (stibnite,  $\text{Sb}_2\text{S}_3$ ), 8.2 Å. Pseudo-doublets due to absorption are noted in pattern 30 (cerargyrite,  $\text{AgCl}$ ), the (200) reflection and in pattern 38 (cobaltite,  $\text{CoAsS}$ ), the (220) reflection.

While the experimental inaccuracies discussed can be remedied (the A.S.T.M. has an organization set up to issue new patterns periodically and correct old ones), there remain inherent limitations to the uniqueness of an established standard pattern. So solution, isomorphism, or structural similarity can contribute to the ambiguity of a standard pattern (see Tables V, VI, and VII). The absence of solid solution in an identified phase should always be checked by qualitative spectroscopic analysis and spot tests. If solid solution is indicated, a sensitive back-reflection technique is required to measure the  $d$ -shifts between standard and sample solution. Ambiguities attributed to isomorphism (see Tables



ed VI) or structural similarity (Table VI) usually are resolvable by spectroscopic analysis, spot tests, or some simple physical criterion such as solubility in water, melting point, etc. The identification of multicomponent mixtures the intrinsic limitations illustrated in Tables V to VII become pronounced because of superposition of lines (see Tables II and XI).

The phases present in an unknown may be formed under rather haphazard and unequilibrated conditions conducive to the formation of defect structures (6, 27, 51, 52). The differences between the diffraction pattern of such a defect structure and that of a normal standard may be subtle and escape the notice of the analyst. In general, structural irregularities in crystalline phases may be detected by changes in intensities of the diffraction lines (as compared to an ordered standard), by an increase of diffuse scattering, by the appearance of broad diffraction ghosts (6), by a broadening of one or more types of reflections, and by a change in the small angle scattering. Serious refinements in the normal powder technique, however, are required to gain information of sufficient reliability to justify an evaluation of the type of randomness in a particular phase. In a careful analysis of an unknown, due consideration should be given to the possible presence of defect structures (see Table VIII). The formation of a continuous range of solid solutions of cocrystallized salts is a rather common occurrence.



Figure 2. Back-Reflection Patterns

taken with unfiltered FeK radiation. Specimen-to-film distance, 50 cm. Sector 1 refers to a mechanical mixture of 98% KI and 2% KBr; sector 2, same mixture crystallized from water; sector 3, same mixture fused. All samples ground to ~200-mesh powders

Table VII. Ambiguities Arising from Structural Similarities

Substance		$d = \text{interplanar spacing; } I/I_1 = \text{relative intensity}$											
Si	$d, \text{\AA}$	3.12	..	1.91	1.63	1.354	1.242	1.104	..	0.958	0.916	..	..
	$I/I_1$	1.00	..	1.00	0.63	0.18	0.25	0.40	..	0.06	0.13	..	..
$\beta\text{-ZnS}$	$d, \text{\AA}$	3.12	2.69	1.91	1.63	1.353	1.242	1.104	1.044	0.957	0.913	..	..
	$I/I_1$	1.00	0.05	0.75	0.50	0.05	0.18	0.15	0.05	0.03	0.04	..	..
CuCl	$d, \text{\AA}$	3.12	2.70	1.91	1.63	1.353	1.240	1.104	1.043	..	..	..	..
	$I/I_1$	1.00	0.08	0.60	0.30	0.06	0.08	0.06	0.04	..	..	..	..
CoO	$d, \text{\AA}$	..	2.45	2.12	1.50	1.281	1.227	1.060	0.975	0.951	0.869	0.819	..
	$I/I_1$	..	0.67	1.00	1.00	0.40	0.40	0.10	0.10	0.30	0.20	0.07	..
$\text{Cu}_2\text{O}$	$d, \text{\AA}$	3.00	2.45	2.12	1.51	1.283	1.228	1.065	0.977	0.953	0.869	0.819	..
	$I/I_1$	0.03	1.00	0.31	0.44	0.31	0.05	0.03	0.05	0.03	0.03	0.03	..
FeO	$d, \text{\AA}$	..	2.47	2.14	1.51	1.293	1.238	1.072	0.984	0.959	..	..	..
	$I/I_1$	..	0.50	1.00	0.63	0.15	0.08	0.03	0.03	0.05	..	..	..
CdO	$d, \text{\AA}$	2.70	2.34	1.65	1.412	1.352	1.171	1.075	..	..	..	..	..
	$I/I_1$	1.00	1.00	1.00	0.75	0.30	0.15	0.30	..	..	..	..	..
$\text{Ag}_2\text{O}$	$d, \text{\AA}$	2.72	2.36	1.67	1.422	1.360	1.179	1.082	..	..	..	..	..
	$I/I_1$	1.00	0.40	0.24	0.16	0.03	0.01	0.02	..	..	..	..	..
Al	$d, \text{\AA}$	2.33	2.02	1.43	1.219	1.168	..	..	..	..	..	..	..
	$I/I_1$	1.00	0.40	0.30	0.30	0.07	..	..	..	..	..	..	..
LiF	$d, \text{\AA}$	2.32	2.00	1.42	1.211	1.160	..	..	..	..	..	..	..
	$I/I_1$	0.67	1.00	0.23	0.03	0.03	..	..	..	..	..	..	..
$\alpha\text{-Ce}$	$d, \text{\AA}$	2.97	2.57	1.815	1.55	1.481	1.288	1.179	..	..	..	..	..
	$I/I_1$	1.00	0.60	0.40	0.40	0.28	0.12	0.16	..	..	..	..	..
LiCl	$d, \text{\AA}$	2.96	2.56	1.81	1.55	1.482	1.283	1.178	..	..	..	..	..
	$I/I_1$	1.00	1.00	0.60	0.32	0.12	0.05	0.12	..	..	..	..	..
$\text{ThO}_2$	$d, \text{\AA}$	3.22	2.80	1.97	1.68	..	1.399	1.280	1.245	1.140	..	..	..
	$I/I_1$	1.00	0.38	0.75	0.88	..	0.13	0.38	0.25	0.38	..	..	..
Ca	$d, \text{\AA}$	3.21	2.80	1.97	1.68	1.61	..	1.28	1.246	1.138	..	..	..
	$I/I_1$	1.00	0.30	0.20	0.20	0.10	..	0.05	0.03	0.05	..	..	..
AgCl	$d, \text{\AA}$	3.20	2.77	1.96	1.67	1.60	1.385	1.270	1.240	1.131	..	..	..
	$I/I_1$	0.40	1.00	0.75	0.20	0.25	0.09	0.06	0.20	0.13	..	..	..

Figure 2 illustrates the sensitivity of the back-reflection method for ascertaining this effect.

CLASSIFICATION OF STANDARDS. Up to the present about 2500 patterns have been cataloged at Dow. When one deals with this number of standards the identification of unknowns by the group classification system (24) is adequate, as has been amply demonstrated at Dow during the past ten years. However, it is of theoretical interest to consider what probable limitations may arise when a comparatively larger number of standards is considered. A statistical study of this problem has been made and is presented here purely as a guide to any modified index that might be considered by the diffraction analyst.

To arrive at a tentative answer to the above query it is instructive to examine how the three most intense diffraction lines (reference lines) for each of the cataloged patterns are distributed with respect to interplanar spacing. Figure 3 shows

Table VIII. Defect Structures

Examples	Diffraction Effects	References
Continuous range of solid solutions $\text{K(I, Br)}$	Merging of $\text{K}\alpha$ doublet on back-reflections	...
Structures with "Leerstellen" $\text{Fe}_{1-x}\text{R}$ (R = O, S, Se, Te) $\text{Na}_x\text{WO}_3$	Occurrence of superstructure lines and anomalous change in lattice constants with $x$	(20-23)
$\text{Mg}_{1-x}\text{Al}_x\text{O}_{4-x}$	Broadening of diffraction lines	...
Pseudomorphism $\text{Mg(OH)}_{2(1-x)}\text{O}_x$	...	(1, 37)
Randomness in layer structures $\text{CdBr}_2$ (Wechselstruktur)	X-ray data can be accounted for on basis of a unit of structure containing a fractional stoichiometric weight	(3, 27, 28, 35, 38, 39, 45)
Micas, clays $n\text{CdCl}_2m\text{Cd(OH)}_2$ , luster carbon	Broadening of diffraction lines, changes in intensities	(27, 28, 38)
Alloy systems with transition structures $\text{Al-Cu}$ , $\text{Al-Ag}$ , $\text{Cu}_3\text{FeNi}_3$ , $\text{AuCu}_3$	Broad diffraction ghosts (6)	(6, 11, 19, 36, 55, 56, 61)







69	KAl(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O	4.29	(50)	4.03	(20)	3.24	(20)	22	1.481	3.78	2.67	1.89	36
70	TiAl(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O	4.32	(30)	2.80	(15)	5.46	(15)	H4n; 738	3.14	3.98	4.23	3.65	68
71	RbAl(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O	4.34	(40)	2.81	(30)	3.28	(15)	738	2.44	3.98	4.23	3.65	68
72	CsAl(SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O	4.35	(40)	2.83	(30)	5.5	(15)	302	2.93	3.58	4.43	7.2	61
73	(OH) <sub>2</sub> Si <sub>2</sub> Al <sub>2</sub> O <sub>7</sub> , metalhalloysite	4.36	(50)	7.46	(30)	2.31	(20)	(46)	2.80	2.33	3.26	4.30	50
74	Al <sub>2</sub> (SO <sub>4</sub> ) <sub>3</sub> ·18H <sub>2</sub> O	4.42	(50)	3.95	(30)	2.48	(20)	20	2.65	5.8	4.43	61	70
75	Al <sub>2</sub> O <sub>3</sub> ·3H <sub>2</sub> O, gibbsite	4.48	(125)	4.39	(62.5)	2.45	(40)	13	2.80	5.4	3.26	2.80	70
76	(NH <sub>4</sub> ) <sub>2</sub> AlF <sub>6</sub>	5.14	(50)	2.23	(25)	2.57	(25)	J2; 8.90 Å	2.31	5.46	4.35	2.83	72
77	AlF <sub>3</sub> ·3/2H <sub>2</sub> O	5.50	(50)	3.86	(25)	3.28	(25)	7	4.17	3.5	3.32	2.40	52
78	(OH) <sub>2</sub> Si <sub>2</sub> Al <sub>2</sub> O <sub>7</sub> ·2H <sub>2</sub> O, halloysite	10.1	(40)	4.48	(20)	1.481	(8)	(46)	3.12	4.0	3.62	3.17	5
79	Bentonite	12.2	(40)	4.48	(20)	3.14	(8)	(46)	3.47	28	3.62	2.09	62
80	(OH) <sub>2</sub> Al <sub>2</sub> Si <sub>2</sub> O <sub>7</sub> ·nH <sub>2</sub> O, montmorillonite	15.2	(40)	4.45	(20)	2.54	(20)	(46)	1.59	2	2.65	1.54	34

Table X. Index to Iron-Containing Substances

Below headings 1, 2, 3 are listed respective reference lines and corresponding intensities for substances of second column. Pattern numbers without parenthetical designation refer to published Dow standards (25). Isomorphism is indicated by a bracket; corresponding structure (16) is stated in column "Pattern". Supplementary index (2, 1, 3, No.) refers to ordered arrangement of second reference line and is used whenever first reference line is obscured by superposition.

No.	Substance	1	2	3	Reference	Pattern	1	2	3	No.	1	2	No.
(1)	FeS <sub>2</sub> , pyrite	1.63	(62.5)	2.42	(30)	443	2.01	1.166	1.428	7	2.18	1.88	20
(2)	(Fe, Ni)S <sub>2</sub>	1.675	(75)	2.77	(25)	23 (26)	2.04	1.180	2.89	9	2.00	1.82	6
3	FeS (1700° K.)	1.77	(100)	3.03	(62.5)	110 (26)	2.05	1.184	2.07	11	2.08	1.80	15
4	FeSi <sub>2</sub>	1.84	(100)	2.37	(62.5)	433	2.07	1.196	1.465	13	2.10	1.82	17
5	Cu <sub>2</sub> FeS <sub>4</sub>	1.93	(100)	3.30	(30)	20 (26), 29 (60)	2.44	1.432	2.02	24	2.71	1.66	48
6	FeSi	2.00	(100)	1.93	(20)	432	2.48	1.455	1.58	26	2.01	1.66	7
7	α-Fe	2.01	(40)	1.66	(15)	401	2.07	1.464	2.59	14	2.08	1.84	8
8	FeAl <sub>3</sub>	2.02	(75)	2.08	(62.5)	402	2.50	1.468	1.60	27	2.05	1.84	11
9	FeAl	2.04	(125)	1.180	(25)	403	2.51	1.476	2.95	29	2.07	1.96	13
10	Fe <sub>1-x</sub> S	2.04	(100)	1.71	(50)	B8 { a = 3.43 Å, c = 5.68 Å }	2.52	1.476	1.61	30	2.96	1.81	60
11	β-Fe (1070° K.)	2.05	(75)	1.184	(25)	A2; 2.90 Å	2.53	1.481	1.61	31	2.95	1.81	59
12	FeS	2.06	(75)	2.97	(25)	442	2.53	1.483	1.61	33	2.97	1.86	26
13	δ-Fe (1700° K.)	2.07	(75)	1.196	(25)	A2; 2.93 Å	2.53	1.484	1.63	32	3.03	1.86	64
14	(63Li <sub>2</sub> Fe <sub>2</sub> O <sub>4</sub> ·37Li <sub>2</sub> TiO <sub>3</sub> )	2.07	(75)	1.464	(25)	(B1); 4.142 Å	2.53	1.488	1.62	34	2.50	1.468	27
15	Taenite, 57.7% Fe, 40.8% Ni, 0.5% P	2.08	(75)	1.80	(10)	A1; 3.60 Å	2.55	1.49	1.62	37	3.07	1.88	65
16	Fe <sub>3</sub> N (1370° K.)	2.09	(40)	2.19	(10)	417	2.55	1.492	1.63	35	2.09	1.88	65
17	γ-Fe (1370° K.)	2.10	(40)	1.82	(10)	A1; 3.63 Å	2.55	1.497	1.63	36	2.52	1.476	30
18	Fe <sub>2</sub> Al <sub>3</sub>	2.11	(62.5)	2.05	(62.5)	404	2.57	1.51	2.47	19	2.53	1.481	31
19	FeO	2.14	(40)	1.51	(25)	425	2.57	1.51	2.57	38	2.53	1.483	33
20	Fe <sub>2</sub> N	2.18	(10)	1.88	(4)	418	2.57	1.54	2.88	39	2.54	1.488	34
21	Fe <sub>2</sub> P	2.23	(17.5)	2.03	(10)	429	2.58	1.54	2.88	40	2.55	1.492	35
22	FeAsS	2.43	(30)	1.82	(8)	12 (26)	2.58	1.54	2.88	43	2.55	1.492	36
23	(FeAs)	2.44	(25)	2.67	(20)	93 (26)	2.65	1.59	2.97	44	2.55	1.497	36
24	FeAl <sub>2</sub> O <sub>4</sub>	2.45	(25)	1.432	(20)	H1; 8.10 Å	2.66	1.61	2.70	47	2.55	1.497	36
25	(Co, Fe)AsS	2.48	(50)	1.455	(20)	67 (26)	2.71	1.66	3.11	1.91	2.57	1.51	66
26	FeO·Cr <sub>2</sub> O <sub>3</sub> ·Al <sub>2</sub> O <sub>3</sub>	2.50	(62.5)	1.468	(62.5)	H1; 8.33 Å	2.86	1.678	1.64	48	2.62	3.08	42
27	FeCr <sub>2</sub> O <sub>4</sub>	2.50	(62.5)	2.84	(20)	51 (26); 47 (60)	2.94	1.71	2.37	55	2.94	4.71	58
28	CuFe <sub>2</sub> O <sub>4</sub>	2.51	(75)	1.476	(30)	H1; 8.42 Å	2.94	1.76	2.97	10	2.73	2.53	50
29	MgFe <sub>2</sub> O <sub>4</sub>	2.52	(75)	2.95	(20)	H1; 8.44 Å	3.02	1.77	2.50	63	2.99	3.65	62
30	NiFe <sub>2</sub> O <sub>4</sub>	2.52	(75)	2.95	(20)	H1; 8.35 Å	3.02	1.80	2.50	15	3.22	1.88	67
31	CoFe <sub>2</sub> O <sub>4</sub>	2.53	(50)	1.61	(25)	H1; 8.38 Å	3.02	1.81	2.54	59	3.22	1.88	67
32	ZnFe <sub>2</sub> O <sub>4</sub>	2.53	(50)	1.481	(40)	H1; 8.42 Å	3.02	1.81	2.54	60	3.22	1.88	67
33	FeFe <sub>2</sub> O <sub>4</sub>	2.53	(62.5)	1.483	(50)	H1; 8.44 Å	3.02	1.82	2.54	61	3.22	1.88	67
34	(Mn, Mg)Fe <sub>2</sub> O <sub>4</sub>	2.54	(75)	1.488	(20)	H1; 8.44 Å	3.02	1.82	2.54	62	3.22	1.88	67
35	CuFe <sub>2</sub> O <sub>4</sub>	2.54	(75)	1.492	(20)	H1; 8.44 Å	3.02	1.82	2.54	63	3.22	1.88	67
36	FeV <sub>2</sub> O <sub>4</sub>	2.55	(75)	1.497	(20)	H1; 8.47	3.03	1.86	2.54	64	3.22	1.88	67
37	(Fe, Zn, Mn)(Fe, Mn) <sub>2</sub> O <sub>4</sub>	2.55	(75)	1.497	(20)	H1; 8.47	3.03	1.86	2.54	65	3.22	1.88	67
38	MnFe <sub>2</sub> O <sub>4</sub>	2.57	(100)	1.51	(40)	H1; 8.54	3.07	1.88	2.54	66	3.22	1.88	67
39	FeAl <sub>2</sub> (SiO <sub>4</sub> ) <sub>2</sub>	2.57	(100)	1.54	(40)	Si1; 11.51 Å	3.22	1.88	2.54	67	3.22	1.88	67
40	(Mg, Fe) <sub>2</sub> Al <sub>2</sub> (SiO <sub>4</sub> ) <sub>2</sub>	2.58	(100)	1.54	(50)	Si1; 11.51 Å	3.22	1.88	2.54	68	3.22	1.88	67
40.5	Fe <sub>1-x</sub> As <sub>2-x</sub>	2.59	(100)	2.32	(20)	16 (26)	3.11	1.91	1.63B	66	3.22	1.88	67
41	FeS <sub>2</sub> ·As <sub>2</sub> S <sub>3</sub>	2.60	(30)	3.62	(17.5)	161	2.75	1.94	2.57	53	2.92	2.22	57
42	CdFe <sub>2</sub> O <sub>4</sub>	2.62	(40)	3.08	(25)	16	2.75	1.94	2.57	53	2.92	2.22	57
43	(K, Na)(Mg, Fe) <sub>2</sub> (AlSi <sub>3</sub> O <sub>10</sub> )(OH) <sub>2</sub> , biotite-phlogopite	2.65	(100)	1.54	(40)	Si1; 11.89 Å	2.11	2.05	2.02	21	2.18	2.46	56
44	Ca <sub>2</sub> (Al, Fe) <sub>2</sub> (SiO <sub>4</sub> ) <sub>2</sub>	2.66	(100)	1.59	(62.5)	407	2.02	2.08	1.445	18	2.86	1.678	55
45	Fe <sub>2</sub> Cl <sub>6</sub>	2.68	(62.5)	2.08	(25)	423	2.09	2.08	2.02	45	2.07	1.464	14
46	Fe <sub>2</sub> O <sub>3</sub>	2.69	(40)	2.51	(30)	Si1; 12.02 Å	2.09	2.22	2.09	16	2.14	1.51	19
47	Ca <sub>2</sub> Fe <sub>2</sub> (SiO <sub>4</sub> ) <sub>2</sub>	2.70	(40)	1.61	(25)	Si1; 12.02 Å	2.92	2.22	2.09	57	6.35	3.30	107

(Continued on page 216)



Table X (Cont.)

No.	Substance	1 Å.	2 Å.	3 Å.	Pattern	Reference	2 Å.	1 Å.	3 Å.	No.	1 Å.	2 Å.	3 Å.	No.
48	(Mn, Fe) <sub>2</sub> O <sub>3</sub>	2.71	1.66	1.42	D5 <sub>5</sub> ; 9.37 Å.	...	2.22	5.13	2.57	91	2.57	2.49	2.49	91
49	FeS <sub>2</sub> , marcasite	2.71	1.76	3.45	96 (26)	...	2.22	5.25	2.63	92	2.63	2.50	2.50	92
50	FeTiO <sub>3</sub>	2.73	2.53	1.72	81 (26)	...	2.32	5.35	2.68	93	2.68	2.50	2.50	93
51	(OH) <sub>2</sub> (Ca, Na, K) <sub>2-3</sub> (Mg, Fe, Al) <sub>3</sub> O <sub>2</sub> , hornblende	2.73	3.7	1.72	...	(46)	2.32	5.35	2.68	40.5	2.68	2.51	2.51	40.5
52	(Na, Al, Ca, Fe) <sub>3</sub> Mn <sub>2</sub> (PO <sub>4</sub> ) <sub>2.5</sub> (OH) <sub>2</sub> , graphite	2.75	3.08	3.12	...	(44)	2.32	2.59	2.52	4	2.52	2.51	2.51	4
53	NaFe(CN) <sub>6</sub>	2.75	3.08	2.51	809	...	2.37	1.84	5.1	79	5.1	2.51	2.51	79
54	FeO <sub>2</sub> ·2H <sub>2</sub> O, phosphosiderite	2.77	1.94	5.7	...	(42)	2.46	4.21	2.18	56	2.18	2.52	2.52	56
55	NasFe <sub>2</sub>	2.81	4.33	4.67	...	...	2.46	2.917	1.87	100	1.87	2.61	2.61	100
56	(Ni, Fe) <sub>2</sub> Si <sub>4</sub>	2.86	3.97	1.982	Cryolite; 813	...	2.51	2.69	1.84	46	1.84	2.62	2.62	46
57	(Fe, Mn)WO <sub>4</sub>	2.917	1.678	2.37	156 (26)	...	2.53	2.73	1.72	50	1.72	2.63	2.63	50
58	K <sub>2</sub> Fe(CN) <sub>6</sub> ·3H <sub>2</sub> O	2.92	2.22	2.09	162 (26)	...	2.54	5.86	5.08	101	5.08	2.68	2.68	101
59	5Cu <sub>2</sub> S <sub>2</sub> (Cu, Fe, Zn)S <sub>2</sub> As <sub>2</sub> S <sub>3</sub>	2.94	4.71	1.71	58 (26)	...	2.55	3.33	7.4	69	7.4	2.70	2.70	69
60	5Cu <sub>2</sub> S <sub>2</sub> (Cu, Fe, Zn)S <sub>2</sub> Sb <sub>2</sub> S <sub>3</sub>	2.95	1.81	1.541	147 (26)	...	2.55	5.1	3.60	89	3.60	2.72	2.72	89
61	K <sub>2</sub> Fe(CN) <sub>6</sub>	2.98	3.60	2.84	150 (26)	...	2.57	3.63	2.06	74	2.06	2.76	2.76	74
62	(Fe, Mn)(Cb, Ta) <sub>2</sub> O <sub>6</sub>	2.99	3.65	2.84	670	...	2.57	3.64	5.1	86	5.1	2.79	2.79	86
63	FeCrS <sub>4</sub>	3.02	1.77	1.72	40 (26)	...	2.62	4.83	5.1	103	5.1	2.79	2.79	103
64	CuFeS <sub>4</sub>	3.03	1.86	2.50	50 (26)	...	2.62	6.04	5.24	104	5.24	2.82	2.82	104
65	(Cu, Fe, Mo, Sn) <sub>4</sub> (S, As, Te) <sub>3-4</sub>	3.07	1.88	1.601	384; 32 (26)	...	2.63	6.07	5.26	23	5.26	2.84	2.84	23
66	Cu <sub>2</sub> S, FeS, SnS <sub>2</sub>	3.11	1.91	1.63B	42 (26)	...	2.67	2.43	1.81	104	1.81	2.87	2.87	104
67	Cu <sub>2</sub> S <sub>2</sub> FeS	3.22	1.88	1.75	41 (26)	...	2.72	1.63	2.42	1	2.42	2.87	2.87	1
68	FeSO <sub>4</sub>	3.25	4.78	1.99	138 (26)	...	2.72	2.45	1.82	25	1.82	2.88	2.88	25
69	β-FeO·OH	3.33	2.55	7.4	435	(9)	2.74	6.33	5.49	105	5.49	2.88	2.88	105
70	FeSO <sub>4</sub> ·H <sub>2</sub> O	3.40	2.81	2.72	82 (26)	...	2.77	1.675	2.49	95	2.49	2.89	2.89	95
71	Fe(SO <sub>4</sub> ) <sub>2</sub> ·H <sub>2</sub> O	3.42	4.85	3.13	...	...	2.81	3.40	2.92	2	2.92	2.94	2.94	2
72	K <sub>2</sub> CuFe(CN) <sub>6</sub>	3.53	6.8	4.75	436	...	2.82	5.7	4.95	98	4.95	2.95	2.95	98
73	CaK <sub>2</sub> Fe(CN) <sub>6</sub>	3.63	2.57	2.06	437	...	2.83	6.55	5.67	108	5.67	2.97	2.97	108
74	Zn <sub>2</sub> Fe(CN) <sub>6</sub> ·3H <sub>2</sub> O	3.64	2.57	3.64	196	...	2.84	6.66	5.77	110	5.77	2.97	2.97	110
75	Pb <sub>2</sub> Fe(CN) <sub>6</sub> ·3H <sub>2</sub> O	3.68	5.4	6.3	971	...	2.89	6.66	5.77	112	5.77	3.00	3.00	112
76	K <sub>2</sub> Fe(CN) <sub>6</sub>	4.08	3.67	2.94	455	...	2.97	2.06	2.61	85	2.61	3.00	3.00	85
77	FeSO <sub>4</sub> (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> ·6H <sub>2</sub> O	4.14	3.09	3.03	669	...	2.98	4.75	2.61	3	2.61	3.00	3.00	3
78	α-FeO(OH), goethite	4.21	3.80	2.70	424	(9, 54)	3.03	1.77	1.95	42	1.95	3.03	3.03	42
79	AgFeS <sub>2</sub>	4.25	2.45	2.79	140 (26)	...	3.08	2.75	2.51	52	2.51	3.13	3.13	52
80	FePO <sub>4</sub> ·2H <sub>2</sub> O, strengite	4.35	3.10	2.51	...	(43)	3.09	4.35	2.51	77	2.51	3.15	3.15	77
81	FeNH <sub>4</sub> (SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O	4.37	7.1	3.73	440	...	3.14	6.0	2.76	80.8	2.76	3.19	3.19	80.8
82	FeCH <sub>3</sub> CO <sub>3</sub>	4.44	5.6	5.8	444	...	3.25	4.25	2.79	102	2.79	3.20	3.20	102
83	Fe(C <sub>6</sub> H <sub>5</sub> OH) <sub>2</sub> (SO <sub>4</sub> ) <sub>2</sub>	4.59	4.16	4.20	428	...	3.30	1.93	3.15	80	3.15	3.23	3.23	80
84	Na <sub>2</sub> Fe(CN) <sub>6</sub> ·NO <sub>2</sub> ·2H <sub>2</sub> O	4.75	2.98	2.89	811	...	3.30	6.35	2.47	5	2.47	3.45	3.45	5
85	FeC <sub>2</sub> O <sub>4</sub> ·2H <sub>2</sub> O	4.75	2.98	2.61	419	...	3.44	5.7	2.76	107	2.76	3.48	3.48	107
86	2Na <sub>2</sub> Fe(C <sub>2</sub> O <sub>4</sub> ) <sub>3</sub> ·9H <sub>2</sub> O	4.83	2.62	5.1	422	...	3.51	4.44	5.8	99	5.8	3.91	3.91	99
87	FeSO <sub>4</sub> ·7H <sub>2</sub> O	4.90	3.78	3.23	438	...	3.51	9.8	5.1	82	5.1	3.51	3.51	82
88	Cu <sub>2</sub> Fe(CN) <sub>6</sub> ·7H <sub>2</sub> O	5.0	3.78	2.50	362	...	3.55	5.0	2.50	88	2.50	3.60	3.60	88
89	Fe[Fe(CN) <sub>6</sub> ] <sub>2</sub>	5.1	2.55	3.60	412	...	3.60	2.98	2.84	61	2.84	3.70	3.70	61
90	Ca <sub>2</sub> Fe(CN) <sub>6</sub> ·12H <sub>2</sub> O	5.13	2.22	4.70	195	...	3.61	6.9	2.18	112	2.18	3.73	3.73	112
91	LiFeFe <sub>2</sub>	5.25	2.28	2.57	(J2); 8.88 Å.	...	3.62	2.60	3.15	41	3.15	3.99	3.99	41
92	(NH <sub>4</sub> ) <sub>2</sub> FeFe <sub>2</sub>	5.25	2.32	2.63	(J2); 9.10 Å.	...	3.62	7.0	3.19	113	3.19	4.20	4.20	113
93	Na <sub>2</sub> Fe <sub>2</sub>	5.35	3.98	3.00	(J2); 9.26 Å.	...	3.65	2.99	1.72	76	1.72	4.28	4.28	76
94	FeCl <sub>2</sub> ·4H <sub>2</sub> O	5.4	3.98	4.28	410	...	3.67	4.10	6.3	87	6.3	4.50	4.50	87
95	FeCl <sub>2</sub> ·2H <sub>2</sub> O	5.5	3.91	4.28	409	...	3.78	4.90	3.23	76	3.23	4.67	4.67	76
96	FeFe <sub>2</sub> ·4H <sub>2</sub> O	5.5	3.91	4.28	413	...	3.80	4.20	3.03	87	3.03	4.70	4.70	87
97	FeSO <sub>4</sub> ·4H <sub>2</sub> O	5.5	4.49	4.95	437	...	3.91	5.5	3.48	96	3.48	4.75	4.75	96
98	Iron ammonium chloride	5.7	2.82	4.95	411	...	3.97	2.81	1.982	54	1.982	5.7	5.7	54
99	Fe(H <sub>2</sub> PO <sub>4</sub> ) <sub>3</sub>	5.7	3.44	2.78	430	...	3.98	5.4	3.00	94	3.00	5.08	5.08	94
100	K <sub>2</sub> Fe <sub>2</sub>	5.73	2.48	2.87	(J2); 9.93 Å.	...	3.99	6.7	3.70	111	3.70	5.1	5.1	111
101	Fe(NH <sub>4</sub> ) <sub>2</sub> Cl <sub>2</sub>	5.86	2.54	5.08	J1; 10.15 Å.	...	3.99	6.6	3.70	111	3.70	5.1	5.1	111
102	FeCl <sub>2</sub> ·6H <sub>2</sub> O	6.0	3.14	2.76	408	...	4.16	4.75	2.89	84	2.89	5.1	5.1	84
103	Fe(NH <sub>4</sub> ) <sub>2</sub> Br <sub>2</sub>	6.04	2.62	5.24	J1; 10.47 Å.	...	4.16	4.75	2.89	84	2.89	5.1	5.1	84
104	Fe(NH <sub>4</sub> ) <sub>2</sub> (ClO <sub>4</sub> ) <sub>2</sub>	6.07	2.63	5.26	J1; 10.52 Å.	...	4.33	2.77	4.67	53.2	4.67	5.1	5.1	53.2
105	Fe(NH <sub>4</sub> ) <sub>2</sub> ·H <sub>2</sub> O	6.2	8.4	7.6	J1; 10.97 Å.	...	4.49	5.5	3.99	97	3.99	5.7	5.7	97
106	γ-FeO·OH	6.33	2.74	5.49	414	...	4.71	2.94	3.99	58	3.99	5.8	5.8	58
107	Fe(NH <sub>4</sub> ) <sub>2</sub> ·H <sub>2</sub> O	6.35	2.83	5.67	J1; 11.34 Å.	...	4.85	3.25	1.99	68	1.99	5.9	5.9	68
108	Fe(NH <sub>4</sub> ) <sub>2</sub> (BF <sub>4</sub> ) <sub>2</sub>	6.55	2.83	5.67	J1; 11.54 Å.	...	5.1	10.0	3.13	71	3.13	5.24	5.24	71
109	Fe(NH <sub>4</sub> ) <sub>2</sub> (SO <sub>4</sub> ) <sub>2</sub>	6.66	3.30	2.47	416	...	5.1	9.8	4.50	116	4.50	5.26	5.26	116
110	Fe(NH <sub>4</sub> ) <sub>2</sub> ·H <sub>2</sub> O	6.7	3.30	5.77	405	...	5.2	4.08	3.64	75	3.64	5.67	5.67	75
111	K <sub>2</sub> Fe(C <sub>2</sub> O <sub>4</sub> ) <sub>2</sub> ·6H <sub>2</sub> O	6.9	3.61	3.19	421	...	5.4	4.59	4.20	90	4.20	6.33	6.33	90
112	K <sub>2</sub> Fe(C <sub>2</sub> O <sub>4</sub> ) <sub>2</sub> ·3H <sub>2</sub> O	7.0	3.62	3.19	420	...	5.5	5.1	4.70	83	4.70	6.33	6.33	83
113	Fe(NH <sub>4</sub> ) <sub>2</sub> (SO <sub>4</sub> ) <sub>2</sub> ·12H <sub>2</sub> O	7.0	9.2	8.2	439	...	6.8	3.53	4.75	72	4.75	7.4	7.4	72
114	Na <sub>2</sub> Fe(CN) <sub>6</sub> ·10H <sub>2</sub> O	7.4	2.04	3.51	810	...	7.1	6.2	3.73	105	3.73	7.6	7.6	105
115	Fe(C <sub>6</sub> H <sub>5</sub> CO <sub>2</sub> ) <sub>2</sub> ·3H <sub>2</sub> O	9.5	5.2	4.50	415	...	8.4	6.2	7.6	114	7.6	10.0	10.0	114
116	Fe(C <sub>6</sub> H <sub>5</sub> CO <sub>2</sub> ) <sub>2</sub> ·3H <sub>2</sub> O	9.8	3.51	5.1	431	...	8.9	7.0	8.2	114	8.2	10.0	10.0	114
117	Fe(C <sub>6</sub> H <sub>5</sub> CO <sub>2</sub> ) <sub>2</sub> ·3H <sub>2</sub> O	9.8	3.51	5.1	431	...	8.9	7.0	8.2	114	8.2	10.0	10.0	114



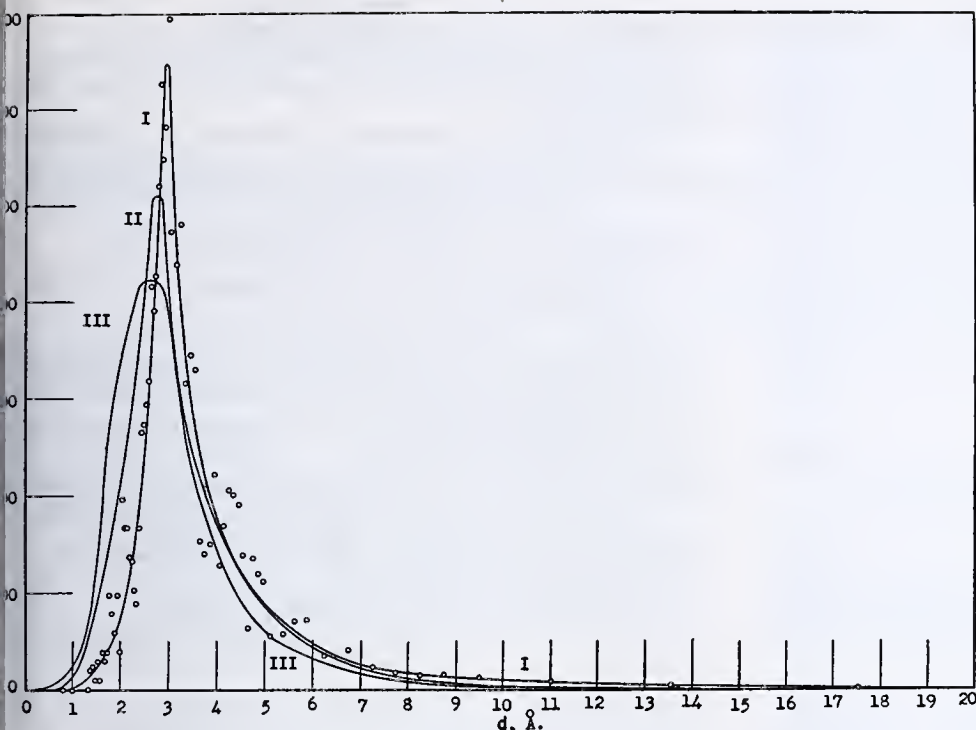


Figure 3. Distribution of Interplanar Spacings of Reference Lines

ers to distribution of first reference line of various standards with respect to  $d$ , interplanar spacing; II and III pertain to second and third reference lines, respectively.  $N_0$  = total number cataloged standards = 2100.  $(dN)_{I,d}$  = number of standards having their first reference located in interval  $(d, \pm \frac{\Delta d}{2})$ . For sake of clarity experimental points are shown only for I. maxima for I, II, and III occur at  $2.95 \pm .08$ ,  $2.75 \pm .20$ , and  $2.60 \pm .30$  Å, respectively.  $n$  = number of diffraction lines per pattern;  $4 \leq n \leq 45$ ;  $\bar{n} = 19$ .  $I_r$  = intensity of  $r^{\text{th}}$  reference line;  $2 \leq \bar{I}_1 \leq 212$  based on  $I_1$  (NaCl) = 150.  $\bar{I}_1:\bar{I}_2:\bar{I}_3 = 42.3:25.7:20.2$  for  $\alpha$  (when corrected for factor  $\frac{1 + \cos^2 2\theta}{\sin^2 \theta \cos \theta}$ , continued ratio is proportional to 26.0:-19.2).  $\nu_1$  expresses probability that  $d_1 > d_2$  or  $d_3$ ;  $\bar{\nu}_1:\bar{\nu}_2:\bar{\nu}_3 = 1.00:50:0.28$ . A bar over a letter signifies an arithmetic average for  $N_0$  standards.

for most common inorganic substances the reference lines within the interval 2.0 to 3.5 Å. Considering the first reference line, one finds 15 patterns in the interval 15 to 20 Å. 74 patterns in the interval 3.00 to 3.00 Å. (of course, the second and third reference lines of substance materially reduce the number of standards to be considered). In the identification of substances, however, a crowding of reference lines in the relatively narrow interval from 2.6 to 3.0 Å. might vitiate an undue amount of searching to locate the sought standard patterns in a very large collection of standards. The point can be visualized by the diffraction pattern of a binary mixture in which there are six reference lines the intensities of which may be denoted by  $I_r$  and  $\zeta_r$  where  $r = 1, 2, 3$ . Assuming (1) that there are no superpositions of lines, that the six most intense reference lines are also the six reference lines for the binary mixture, and that  $I_1 \geq I_2 \geq I_3 \geq \zeta_1 \geq \zeta_2$ , one may have to exhaust 1 to 119 possible combinations to find the appropriate set

of reference lines for one component [for an  $n$ -component mixture there are  $3n(3n-1)(3n-2) - (n-1)$  combinations]. In the general case of a binary mixture, allowance must also be made for the following possibilities: (1) the superposition of reference lines, for example, the second reference line of phase A and the third reference line of phase B so that  $I_2 + \zeta_3 > I_r$  or  $\zeta_r$ ; (2) the superposition of a reference line of phase A and a moderately intense reflection of phase B ( $I_3 + \zeta_4 > I_2$ ); and (3) the presence of prominent nonreference lines for phase A, so that  $I_4$  or  $I_4 + \zeta_n > \zeta_2$ . These considerations are general and apply to any scheme of classification.

In case the number of standards exceeds 10,000 to 20,000, the problem may arise as to how to circumvent the probable congestion of reference lines and the multiplicity of trial reference lines for multi-component mixtures. In view of the fact that the great majority of analyses are supplemented or confirmed by qualitative spectroscopic analysis, it may be expedient to reverse the procedure and obtain the spectroscopic data first to facilitate finding the appropriate diffraction standards.

For example, all compounds containing aluminum would be grouped under aluminum as shown in Table IX. Those substances containing both aluminum and iron would be listed under

Table XI. Powder Diffraction Data

Filtered MoK $\alpha$  used to obtain diffraction patterns.  $d$  = interplanar spacing.  $I$  = peak intensity of a diffraction line.  $I/I_1$  = relative intensity, where  $I_1$  is intensity of strongest line of particular phase in question (values in parentheses refer to calculated intensities). Diffraction pattern of albite, Amelia Court House, Va., was taken with a 0.1-mm. slit to resolve some of the broader reflections.

SPECTROSCOPIC ANALYSIS OF UNKNOWN. Fe, Al, Si, Na chief constituents; minor constituents P 0.1 to 0.5%, K 0.01 to 0.1%, Ca 0.001 to 0.01%.

										NaAlSi <sub>3</sub> O <sub>8</sub> , Albite, Amelia Court House, Va.	
Un- known <i>d</i> , Å.	<i>I</i>	Albite, NaAlSi <sub>3</sub> O <sub>8</sub> <i>d</i> , Å.	<i>I</i> / <i>I</i> <sub>1</sub>	Phase I <i>I</i> / <i>I</i> <sub>1</sub>	Goethite, α-FeO.OH <i>d</i> , Å.	<i>I</i> / <i>I</i> <sub>1</sub>	Phase II <i>I</i> / <i>I</i> <sub>1</sub>	Σ <i>I</i>	<i>d</i> , Å.	<i>I</i> / <i>I</i> <sub>1</sub>	
6.5	1	6.4	0.08	0.02	..	..	..	..	6.4	0.06	
5.0	3	..	..	..	4.98	0.04	0.09	..	5.9	0.04	
4.20	25	..	..	..	4.21	1.00 (25)	0.76	..	5.5	0.02	
4.02	12.5	4.05	0.35	0.28	..	..	..	..	4.01	0.50 (50)	
3.80	3	3.80	0.16	0.07	..	..	..	..	3.84	0.06	
3.67	9B	3.66	0.25	0.20	..	..	..	..	3.76	0.08	
3.38	4	..	..	..	3.39	0.12	0.12	..	3.66	0.40 (40)	
3.19	50	3.20	1.00 (50)	1.14	..	..	..	..	3.50	0.10	
2.95	12.5	2.96	0.25	0.28	..	..	..	..	3.37	0.08	
2.69	12.5	2.65	0.02	(1)	2.70	0.36	0.38	..	3.19	1.00B (100)	
2.57	12.5	2.56	0.12	(5)	2.58	0.24	(8)	13	2.94	0.20B	
2.44	30	2.44	0.14	(6)	2.45	0.80	(26)	32	2.85	0.10	
2.37	1	..	..	..	..	..	..	..	2.64	0.04	
2.32	2	2.32	0.12	0.05	..	..	..	..	2.55	0.10	
2.25	5	..	..	..	2.25	0.12	0.15	..	2.43	0.10	
2.18	6	2.18	0.06	(3)	2.19	0.20	(7)	10	2.38	0.04	
2.10	3B	2.13	0.12	0.07	..	..	..	..	2.31	0.10B	
2.01	4	1.99	0.08	0.09	..	..	..	..	2.24	0.01	
1.90	5B	1.90	0.12	(5)	1.92	0.08	(3)	8	2.19	0.02	
1.81	6B	1.83	0.18	(8)	1.80	0.08	(3)	11	2.12	0.08	
1.72	17.5	1.73	0.08	(4)	1.72	0.36	(12)	16	2.06	0.06	
1.60	4	..	..	..	1.60	0.08	0.12	..	1.98	0.06B	
1.56	15	1.58	0.12	(5)	1.56	0.28	(9)	14	1.89	0.10	
1.51	12.5	1.50	0.08	(4)	1.50	0.24	(8)	12	1.85	0.08	
1.455	10	1.460	0.16	(7)	1.455	0.12	(4)	11	1.82	0.10	
1.419	9	1.425	0.16	(7)	1.420	0.04	(1)	8	1.782	0.08	
1.350	4	1.350	0.14	(6)	1.355	0.08	(3)	9	1.743	0.04	
				( <i>I</i> <sub>1</sub> ) <sub>I</sub> ≈ (44)					( <i>I</i> <sub>1</sub> ) <sub>II</sub> ≈ (33)	1.720	0.04



aluminum and iron—e.g.,  $\text{FeAl}_2\text{O}_4$  is listed in Tables IX and X (there are nine such substances listed). Under the nonmetals—H, C, N, O, F, S, Cl, Se, Br, Te, I—one would list only those compounds which do not contain elements detectable by the conventional arc spectra—i.e., which do not contain any of the following: Li, Be, B, Na, Mg, Al, Si, P, K, Ca, Sc, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Ga, Ge, As, Rb, Sr, Y, Zr, Nb, Mo, Ru, Rh, Pd, Ag, Cd, In, Sn, Sb, Cs, Ba, La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Lu, Hf, Ta, W, Re, Os, Ir, Pt, Au, Hg, Tl, Pb, Bi, Ra, Th, U. Chemical tests are convenient to identify the nonmetals—e.g., the use of aqueous sodium hydroxide and Nessler's reagent to detect ammonium salts. Organic compounds may also be detected in the arc (using copper-electrodes) by the 2478.5 Å. emission line of carbon and the cyanogen band. For routine analysis it would be advisable to check the diffraction standards listed under carbon and nitrogen.

The procedure for identifying an unknown by this modification of the triple index system (25) is illustrated by Table XI.

Qualitative spectroscopic analysis of the unknown shows the following chief constituents: iron, aluminum, silicon, sodium. The more prominent diffraction lines of the unknown (column I, Table XI) are: 4.20, 4.02, 3.19, 2.95, 2.69, 2.57, 2.44, 1.72 Å. Looking under Table X to locate the iron-containing phase(s), one finds  $\alpha\text{-FeO}(\text{OH})$  as a likely component of the unknown. Checking the complete data of standard pattern 424, one establishes the presence of goethite in the mixture. In a like manner Table IX indicates albite,  $\text{NaAlSi}_3\text{O}_8$ , as the second phase of the unknown. The presence of albite also could have been ascertained by looking under the index for sodium or silicon. As all the diffraction lines of the unknown are satisfactorily accounted for, the qualitative compound analysis is considered complete and there is no need to check the diffraction standards listed under carbon, nitrogen, etc.

The extent to which the suggested classification of diffraction standards by elements circumvents the anticipated congestion of reference lines can be estimated by an examination of Tables IX and X in regard to the number of times the average compound is listed under more than one element.

For aluminum (Table IX) there are 18 substances listed only once (under aluminum), 47 substances listed twice (distributed over 23 elements), 11 substances listed three times (distributed over 8 elements), and two substances listed 4 times (distributed over 5 elements). For Table X there are 39 substances indexed only under iron, 60 substances indexed twice (distributed over 23 elements), 13 substances listed three times (distributed over 19 elements), and five minerals listed 4 times (distributed over 11 elements). A calculation of the quotient

$$\frac{\sum n_r}{N \sum n_r}$$

where  $n_r$  = number of substances listed  $r$  times  
 $N$  = total number of elements under which the various substances are indexed

shows that the congestion of reference lines has been reduced by a factor of 12.7 for the aluminum compounds and 13.9 for the iron compounds.

However, it appears that the intrinsic advantage of combining spectroscopic information with diffraction data is the natural incorporation of isomorphous groups in the various tables. This combination of empirical standards and structural types into one index should broaden the scope and utility of chemical analysis by powder diffraction.

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# Potentiometric Determination of Acidity in Highly Colored Materials

## Application to New and Used Petroleum Lubricants Containing Additives

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Potentiometric methods for the determination of free and combined acidity of materials soluble only in a nonaqueous solvent are presented; they are particularly applicable where acidimetric color indicators fail—that is, to highly colored or opaque materials such as used lubricants or lubricants containing oxidation corrosion inhibitors, detergents, fats, and other additives. Although developed primarily for study of the oxidation characteristics of lubricating oils, the principles, apparatus, and procedures are applicable to many other materials such as asphalt, emulsions, resins, polymers, animal and vegetable fats, oils, etc.

For the determination of acidity in highly colored or opaque materials methods that depend upon indicator color change are inadequate and often useless, even though they may be applicable to colorless materials insoluble in water or ethyl alcohol. A potentiometric method for the determination of neutralization end points, the logical alternative, seems especially promising when considered in conjunction with a titration solvent that is capable of dissolving or dispersing a suitable portion of water-insoluble material. While the present discussion deals with petroleum products such as heavy oils, asphalts, resins, and the like, the principles are equally applicable to many other commercial materials.

A potentiometric method which is both sound in principle and easily applicable in practice should combine several important characteristics. The electrodes should be sturdy and readily give reproducible values in identical solutions, but should not be attacked by dilute acid or base solutions or organic materials, be subject to atmospheric oxidation, or appreciably contaminate the titration solvent. The potential difference of the electrode system should be nearly proportional to the hydrogen-ion activity of the mixture of titration solvent and sample, and equilibrium should be attained in a conveniently short time. The titration solvent should completely dissolve an adequate amount of sample and the mixture should tolerate the presence of several milliliters of water from the sample without formation of a second phase. The titration medium should have a low inherent acid value, and should be inert to prolonged action of strong bases, strong acids, metals, glass, and atmospheric materials. The polar properties of the solvent should be such that dissolved acidic materials, with dissociation constants greater than  $10^{-7}$  when dissolved in water, will ionize sufficiently to permit neutralization by the addition of an equivalent quantity of an alcoholic strong base solution. The titration solvent should be sufficiently conductive to allow only momentary accumulation of electrostatic charges when a low-resistance reference electrode is immersed in it.

While there have been many noteworthy contributions on the potentiometric titration of acids in nonaqueous solutions (1-37), no published method entirely fulfills the above conditions.

The hydrogen electrode is slow, troublesome, and easily poisoned by a variety of materials (8, 32). Although the quinhydrone electrode has found repeated application in nonaqueous solutions (5, 6, 11, 13, 19, 30, 31, 35), it cannot be used in all solvents; it is attacked by dissolved oxygen, and is not reliable in alkaline solutions. The antimony electrode functions well as an indicating electrode (20), but is not reproducible from time to time and is not universally applicable; this is generally true of

all similar metal electrodes (33). Platinum-tungsten (28), platinum-carbon (27), and other dissimilar electrode pairs (4, 9) function only in systems that show distinct, sudden changes in hydrogen-ion activity; they are easily influenced by extraneous materials. The thin unshielded glass electrode (7, 12, 14, 29) is reproducible and accurate, but undesirably fragile. The modern high-resistance glass electrode has ample mechanical strength, but requires adequate shielding in order to avoid error due to electrostatic influences, and is not applicable in completely anhydrous solvents (12).

The saturated calomel electrode, in various forms, is generally desirable as a reference electrode when used with a suitable salt bridge. The agar-agar salt bridge is appreciably attacked by organic solvents and must be renewed at frequent intervals (6, 11, 29, 30). The aqueous salt bridge with a ground-glass joint contact is generally reproducible, if properly prepared and maintained. The use of a nonaqueous salt bridge is feasible (3, 12, 16, 20, 30) and desirable, but further work is needed to establish satisfactorily the application of the nonaqueous reference electrode. In some instances, the silver-silver chloride electrode (11, 12, 13, 31) is a satisfactory reference but it is very slow in attaining equilibrium and gives a distorted potential-volume titration curve.

The potentiometric methods given in this paper have proved satisfactory for determining the free and combined acidic, or basic, constituents present in new or oxidized petroleum oils and in other petroleum products. The free acidity, or basicity, is determined by potentiometrically titrating the sample dissolved in a benzene-isopropyl alcohol solution, using a glass-calomel electrode system. The combined acidity is similarly determined by potentiometric titration with alcoholic acid after saponifying the sample dissolved in a benzene-isopropyl alcohol solution containing an excess of strong base. These methods are applicable to materials that are soluble, or nearly soluble, in benzene-isopropyl alcohol mixtures, and that are colorless, colored, or produce colored solutions during the determination. Unchanged compounds that are only weakly acidic or basic, and whose dissociation constants,  $K_a$  or  $K_b$ , in water are equal to or less than  $K = 10^{-9}$ , are not detectable and do not interfere. Various acid or base groups may be distinguished, provided there is a satisfactory difference between the dissociation constants of the groups. Thus strong acids, such as hydrochloric, are distinguishable from weak acids, such as formic and acetic, which in turn are distinguishable from weaker acidic materials such as thiophenol. Similar relationships hold for basic groups of like differences in basicity. Covering a period of four years, these methods have been successfully used for the determination of free and combined acidity in new and oxidized motor oils, turbine oils, oil additives, motor oil sludges, asphaltene, crude oils, asphalt, asphalt resi-



dues, distillates, distillate bottoms, polymers, rubber, soaps, vegetable and animal oils, fats, waxes, greases, common solvents, and water solutions.

The applicability of the glass electrode to the measurement of hydrogen-ion activity in nonaqueous solutions has been frequently questioned from a theoretical viewpoint. However, titration curves made in nonaqueous solution using the glass-calomel electrode pair are perfectly reproducible, and are usable for accurately measuring the acid or base content of the solutions. To avoid confusion of titration data obtained in nonaqueous media with those obtained in water, a new unit, the *cG* unit, has been coined to replace the pH unit in nonaqueous media.

It has been found (10, 17) that the potential of a glass electrode in aqueous media depends upon the hydrogen-ion activity of the solution according to the equation (at 25° C.):

$$E_G = E_G^\circ + 0.0591 \log (H^+ \text{ activity})$$

Assuming that the potential of the glass electrode varies in a similar manner with the hydrogen-ion activity in nonaqueous solution, the following equation can be considered valid:

$$E_G = E_G^\circ + cG$$

The value of  $E_G^\circ$  for any particular glass electrode is constant. Under suitable conditions and assuming that liquid junction effects are negligible, the potential of a saturated calomel electrode is constant, and not dependent upon the hydrogen-ion activity or upon the activity of any other dissolved substance. Therefore

$$E = E_G^\circ + E_{GC}^\circ + cG$$

$$E = E_{GC} + cG$$

where  $E$  is the measured electromotive force between the electrodes and  $E_{GC}$  is a constant which must be determined by calibration for each electrode pair.

### Methods for Free and Combined Acid and Base Numbers

Since complete details of these methods are available (1, 2), only a brief outline is included here.

#### APPARATUS

The following apparatus has been found through experience to be most satisfactory:

**METER.** The electronic voltmeter (26), the dual alternating current titrometer described by Penner and Rolfsen (25), or the Beckman model M or Model O industrial pH meters.

**ELECTRODES.** High-resistance glass electrode No. 4990, and the sleeve-type calomel electrode, No. 4970A, manufactured by the National Technical Laboratories, and supplied on Beckman meters.

**TITRATION STAND,** described by Lykken and Rolfsen (21).

**REFLUXING APPARATUS.** Regular saponification apparatus including hot plate, Allihn-type condenser, and 300-ml. Erlenmeyer flask, or the special apparatus pictured in Figure 3 of (2).

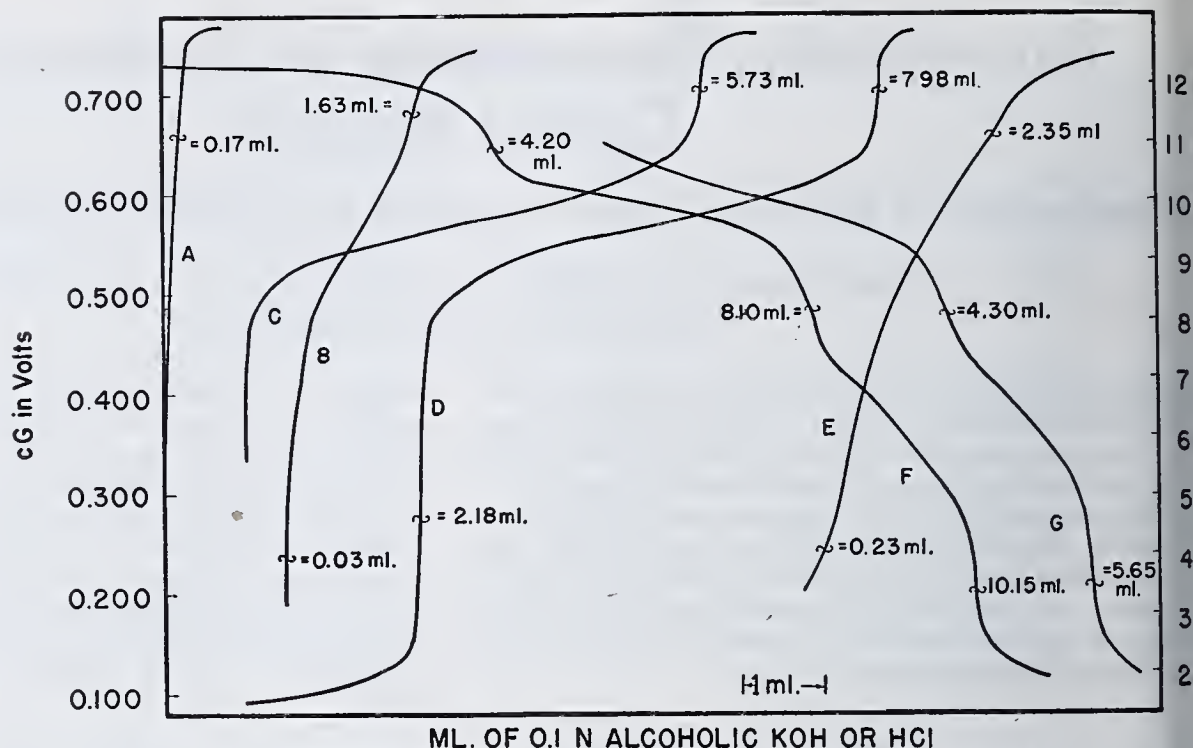


Figure 1. Illustrative Titration Curves for Determination of Free Acids and Bases

Potentiometric titration with base

- A. Blank
- B. 10 grams of oxidized oil
- C. Naphthenic acids
- D. Hydrochloric and naphthenic acids
- E. 5 grams of asphalt

Potentiometric titration with acid

- F. 5 grams of alkaline oil containing free caustic
  - G. 5 grams of alkaline oil
- Titration cell. Glass electrode || saturated calomel electrode  
Titration solvent. 50 ml. of benzene and 50 ml. of isopropyl alcohol containing 1% water

#### REAGENTS

Standard 0.1 *N* and 0.2 *N* potassium hydroxide in isopropyl alcohol. Standard 0.1 *N* and 0.2 *N* hydrochloric acid in isopropyl alcohol. Standard aqueous pH = 4 buffer. Standard aqueous *cG* = 0.650-volt buffer (2). Standard nonaqueous *cG* = 0.236-volt buffer (2).

**TITRATION SOLVENT.** Mixture of equal volumes of c.p. benzene and c.p. isopropyl alcohol containing 1% water.

#### CHARACTERISTICS OF ELECTRODE SYSTEM

The glass-calomel system requires constant and regular attention to obtain reproducible values. After every titration electrodes must be rinsed thoroughly with benzene-isopropyl alcohol and water, carefully dried with a clean towel, and so for a short time in distilled water. Special care must be taken in preparing the ground-glass sleeve of the calomel electrode.

Before use, the electrode pair is standardized with aqueous pH = 4 buffer, and calibrated with two nonaqueous buffers *cG* = 0.650 and 0.236 volt. The constant  $E_{GC}$  is calculated by the expression:

$$E_{GC} = E - 0.236$$

where  $E$  is the voltage of the cell when immersed in pH = 4 buffer.

#### PROCEDURE FOR FREE ACID (ACID NO.)

Weigh or pipet a sample of less than 20 grams containing 0.2 to 0.5 milliequivalent of acid into a tall-form 250-ml. titration beaker, add 100 ml. of titration solvent, and adjust the electrodes so that they are immersed in the solvent. Titrate with 0.1 *N* alcoholic potassium hydroxide, making certain sufficient time is allowed for equilibration between increments of caustic. A satisfactory indication of equilibrium is a cell voltage "drift" of less than 5 millivolts per minute. Plot a graph of millivolts of potassium hydroxide as abscissas, and millivolt pH scale readings as ordinates. Mark inflections that occur in the neighborhood of *cG* = 0.236 and 0.650 volt (corresponding to pH scale readings of 4.0 and 11.0, respectively). Determine the number of milliliters required to each break. Make a titration following the above directions, but omitting the standardization.

When the titration curve of the sample shows no definite inflection point, the number of milliliters of potassium hydroxide used to obtain *cG* readings of 0.236 and 0.650 volt are taken as equivalent to the amounts of strong and total acids, respectively. The voltages between the electrodes corresponding to *cG* = 0.650 and 0.236 volt, as calculated using the constant  $E_{GC}$



used as the correct values for any series of determinations with a given electrode system. For work of a special nature or cooperative or referee testing, the voltages corresponding to the  $cG$  values should be obtained by calibration with standard aqueous buffers.

#### PROCEDURE FOR FREE BASE (BASE NO.)

Proceed exactly as for free acid, using 0.1 *N* hydrochloric acid instead of potassium hydroxide.

#### CALCULATIONS FOR FREE ACID AND FREE BASE

Figure 1 gives acid and base titration curves which illustrate typical shapes commonly encountered and the manner of selecting end points. The amount of base needed to neutralize acids up to the first end point near  $cG = 0.236$  volt less the equivalent blank titration is a measure of the acid constituents in strong acid characteristics. The amount of base needed to neutralize the acids between the end points near  $cG = 0.236$  and  $0.650$  volt less the equivalent blank titration is a measure of the acid constituents with weak acid characteristics. Basic materials neutralized by hydrochloric acid above the end point near  $cG = 0.650$  are strong bases and those neutralized between the end points near  $cG = 0.650$  and  $0.236$  volt are weak bases.

#### PROCEDURE FOR COMBINED ACIDS AND BASES (SAPONIFICATION NO.)

**GENERAL PROCEDURE.** Although this procedure is the most general one, it is used only for samples of unknown saponification characteristics or for samples that upon saponification produce an adherent precipitate which clings to the surface of the reflux vessel. The simplified direct titration procedure is generally preferred whenever it is applicable because it allows a considerable saving of time and manipulative effort.

Weigh or pipet a sample containing from 1 to 3 milliequivalents of combined acid or base into a suitable reflux vessel. Add boiling

chips, 40 ml. of c.p. benzene, and 40 ml. of 0.2 *N* alcoholic potassium hydroxide. Reflux gently for 2 hours, remove from the hot plate, cool, and transfer the contents to a titration beaker. Adjust the beaker so that the electrodes are immersed in the solvent, and titrate with 0.2 *N* alcoholic hydrochloric acid as directed in free base determination. Select the strong base and weak base end points in the same way as for free base (above). To recover caustic or weak bases left adhering to the walls of the reflux vessel, add 100 ml. of water, cover the vessel, and bring to a boil. Cool the water solution, pour into a titration beaker, place the beaker upon the titration stand, and titrate with 0.2 *N* alcoholic hydrochloric acid. On the water titration curve select the end points near the  $cG$  potentials of 0.49 and 0.30 volt (pH 8.2 and 5.0, respectively). Add the number of milliliters of hydrochloric acid required for the upper break in the aqueous titration curve to the number of milliliters required for the upper break in the nonaqueous titration curve, and the number of milliliters for the lower break in the aqueous titration curve to the corresponding number in the nonaqueous titration curve.

Make a blank determination using the above procedure, but omitting the sample.

**DIRECT TITRATION PROCEDURE.** Carry out the determination as directed in the general procedure, but add sample, caustic, and benzene directly to the titration beaker, reflux in an apparatus with an immersion-type condenser, cool, and titrate directly in the reflux beaker.

#### CALCULATIONS FOR COMBINED ACIDS AND BASES

Figure 2 gives typical combined acid-base titration curves which illustrate the shapes commonly encountered and the method of selecting end points. Where no definite breaks are obtained, the arbitrary points at which  $cG = 0.650$  and  $0.236$  volt are selected as in the free acid procedure.

The difference in the amount of standard acid required to produce the end point near  $cG$  potential 0.650 volt (pH scale reading of 11.0) for the sample and the blank determinations is a measure of the amount of free and combined acidic constituents in the

sample; this difference is equivalent to the customary saponification number. The difference in the amount of standard acid required to produce the end point near  $cG$  potential of 0.24 volt (pH scale reading of 4.0) for the sample and blank determinations is a measure of the free and combined strong acid constituents in the sample if the blank titration is greater than the sample titration; if the sample titration is greater than the blank titration, the difference between them is a measure of the basic constituents present in the sample. These relationships do not apply if the sample contains strong acids or bases, along with constituents that upon saponification produce bases or acids, respectively.

In those cases in which a water solution is prepared and titrated, the amount of standard acid used to produce an end point near  $cG$  potential of 0.485 volt (corresponding to pH 8.2) in the water solution titration is added to that required to produce an end point near a  $cG$  potential of 0.650 volt (corresponding to pH scale reading of 11.0) in the benzene-isopropyl alcohol solution

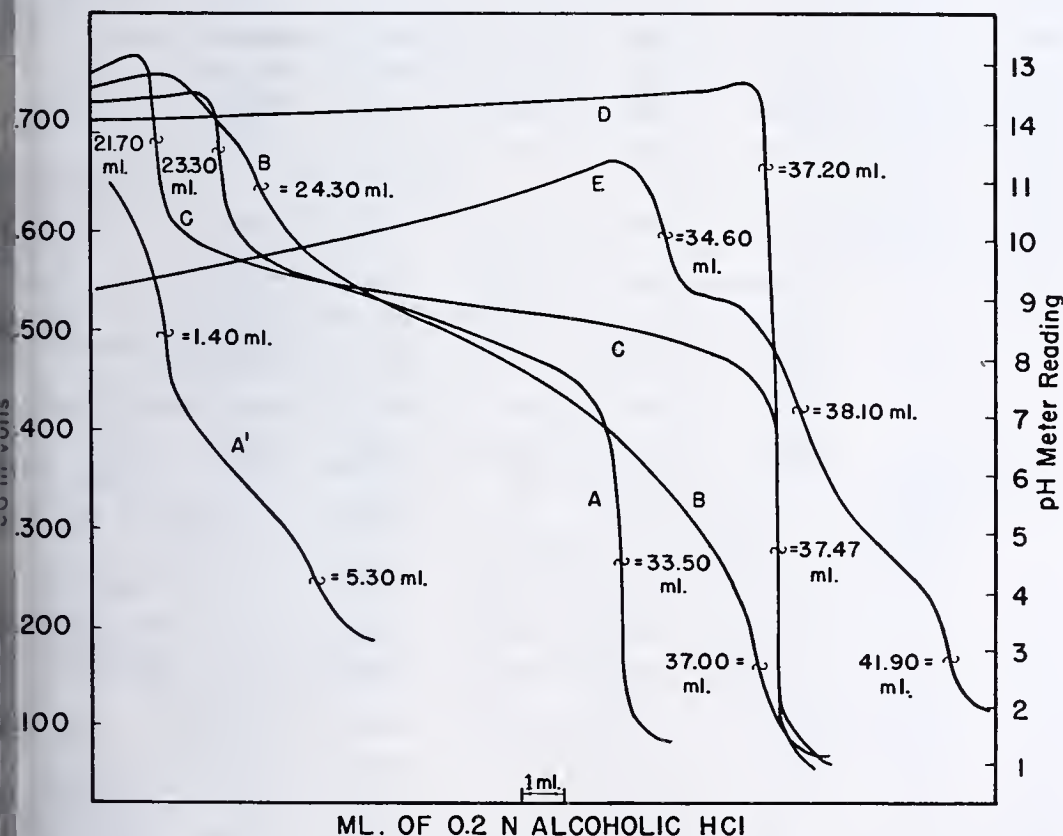


Figure 2. Illustrative Titration Curves for Determination of Combined Acids and Bases

Potentiometric titration with alcoholic hydrochloric acid of basic constituents remaining after saponification with potassium hydroxide

- A. Rapeseed oil, titration of nonaqueous saponification mixture
- A'. Rapeseed oil, titration of water-soluble residue remaining in saponification vessel
- B. Oxidized oil, titration of nonaqueous saponification mixture directly in reflux vessel
- C. Castor oil, titration of nonaqueous saponification mixture directly in reflux vessel
- D. Blank, titration of nonaqueous saponification mixture directly in reflux vessel
- E. Alkaline oil, titration of nonaqueous saponification mixture directly in reflux vessel

Titration cell. Glass electrode || calomel electrode

Saponification medium. 50 ml. of benzene, 50 ml. of Isopropyl alcohol



titration. Similarly, the amount of standard acid used to produce an end point near *cG* potential of 0.295 volt (corresponding to pH 5.0) in the aqueous titration is added to that required to produce an end point near a *cG* potential of 0.236 volt (corresponding to pH scale reading of 4.0) in the nonaqueous titration. These sums are used to calculate the various differences and the calculations are then made as though only one titration curve had been obtained.

### ACCURACY AND PRECISION

The accuracy and precision of the free acidity method depend upon the acids present, and upon the materials associated with the acids. In the determination of naphthenic acids in 10 gram of lubricating oil, the standard deviation is less than 0.05 ml. 0.1 *N* alkali, and the systematic error is less than  $\pm 1.96$  times the standard error.

The accuracy and precision of the combined acidity method

Table I. Electrochemical Characteristics of Various Nonaqueous Solvents

Solvent Composition	Water Added, Ml.	Electrodes Indicating	Reference	Solvent Action for Heavy Oil	Equilibrium Rate of Electrodes	Electrical Conductivity	Inherent Acidity of Solvent	Chemical Stability of Solvent	Nature of Inflection Point	Remarks
100 ml. (CH <sub>3</sub> ) <sub>2</sub> CHOH	10	Glass	Calomel	Poor	Very good	Good	Low	Inert	Excellent	Standard of reference
100 ml. (CH <sub>3</sub> ) <sub>2</sub> CHOH	1	Glass	Calomel	Fair-poor	Very good	Fair	Low	Inert	Excellent	
100 ml. (CH <sub>3</sub> ) <sub>2</sub> CHOH	None	Glass	Calomel	Fair-poor	Good	Fair	Low	Inert	Excellent	Equilibrium slow break
15 ml. CHCl <sub>3</sub> + 90 ml. (CH <sub>3</sub> ) <sub>2</sub> CHOH	1.5	Sb	Calomel	Good	Good	Good	Low	Slightly reactive	Good	
25 ml. CHCl <sub>3</sub> + 75 ml. (CH <sub>3</sub> ) <sub>2</sub> CHOH	0.75	Glass	Calomel	Very good	Good	Good	Low	Slightly reactive	Excellent	Little acid formed during titration
50 ml. CHCl <sub>3</sub> + 100 ml. (CH <sub>3</sub> ) <sub>2</sub> CHOH	1	Glass	Calomel	Good	Good	Good	Low	Reactive	Good	Little acid formed during titration
50 ml. CHCl <sub>3</sub> + 100 ml. (CH <sub>3</sub> ) <sub>2</sub> CHOH	1	Sb	Calomel	Good	Good	Very good	Low	Reactive	Good	LiCl added
75 ml. CHCl <sub>3</sub>	None	Sb	Calomel	Very good	Slow	Very poor	Low	Reactive	Good	Acid formed during titration
75 ml. CHCl <sub>3</sub>	0.015	Sb	Calomel	Excellent	Slow	Good	Low	Very reactive	Poor	LiCl added, m base consumed
75 ml. CHCl <sub>3</sub> + 25 ml. (CH <sub>3</sub> ) <sub>2</sub> CHOH	None	Sb	Calomel	Very good	Slow	Fair	Low	Reactive	Very poor	Acid formed during titration
75 ml. CHCl <sub>3</sub> + 35 ml. (CH <sub>3</sub> ) <sub>2</sub> CHOH	0.1	Glass	Calomel	Very good	Slow	Good	Low	Reactive	Very poor	Acid formed during titration
100 ml. CHCl <sub>3</sub> + 10 ml. (CH <sub>3</sub> ) <sub>2</sub> CHOH	1	Glass	Calomel	Very good	Slow	Poor	Low	Reactive	Very poor	Acid formed during titration
75 ml. CCl <sub>4</sub>	None	Sb	Calomel	Excellent	Slow	Very poor	Low	Very reactive	Good	Acid formed during titration
75 ml. CCl <sub>4</sub> + 35 ml. (CH <sub>3</sub> ) <sub>2</sub> CHOH	1	Glass	Calomel	Very good	Slow	Poor	Low	Reactive	Fair	
75 ml. CCl <sub>4</sub> + 35 ml. (CH <sub>3</sub> ) <sub>2</sub> CHOH	1	Sb	Calomel	Very good	Slow	Poor	Low	Very reactive	Poor	Acid formed during titration
100 ml. C <sub>6</sub> H <sub>6</sub>	None	Glass	Calomel	Excellent	.....	Nil	.....	.....	.....	Nonconducting, titration impossible
50 ml. (CH <sub>3</sub> ) <sub>2</sub> CHOH + 50 ml. C <sub>6</sub> H <sub>6</sub>	None	Glass	Calomel	Excellent	Good	Poor	Low	Inert	Excellent	
50 ml. C <sub>6</sub> H <sub>6</sub> + 50 ml. C <sub>2</sub> H <sub>5</sub> OH	0.5	Glass	Calomel	Good	Good	Fair	Low	Inert	Good	
75 ml. C <sub>6</sub> H <sub>6</sub> + 75 ml. (CH <sub>3</sub> ) <sub>2</sub> CHOH	0.8	Glass	Calomel	Excellent	Good	Fair	Low	Inert	Excellent	
75 ml. C <sub>6</sub> H <sub>6</sub> + 25 ml. (CH <sub>3</sub> ) <sub>2</sub> CHOH	None	Glass	Calomel	Excellent	Slow	Very poor	Low	Inert	Excellent	
75 ml. C <sub>6</sub> H <sub>6</sub> + 25 ml. (CH <sub>3</sub> ) <sub>2</sub> CHOH	0.3	Glass	Calomel	Excellent	Good	Poor	Low	Inert	Excellent	
100 ml. petroleum ether	None	Glass	Calomel	Good	.....	Nil	.....	.....	.....	Nonconducting, titration impossible
75 ml. petroleum ether + 75 ml. (CH <sub>3</sub> ) <sub>2</sub> CHOH	0.8	Glass	Calomel	Excellent	Good	Fair	Low	Inert	Good	
75 ml. <i>n</i> -butyl alcohol	None	Glass	Calomel	Poor	Very slow	Good	High	Reactive	Good	
75 ml. acetone	0.8	Glass	Calomel	Good	Good	Good	Very high	Inert	Good	
75 ml. <i>n</i> -butyl alcohol	0.8	Glass	Calomel	Good	Good	Very good	Very high	Inert	Good	LiCl added
75 ml. <i>n</i> -butyl alcohol + 75 ml. (CH <sub>3</sub> ) <sub>2</sub> CHOH	0.8	Glass	Calomel	Good	Good	Very good	Very high	Inert	Good	
75 ml. cyclohexanol + 75 ml. (CH <sub>3</sub> ) <sub>2</sub> CHOH	0.8	Glass	Calomel	Good	Slow	Poor	High	Inert	Good	Base dilution "normal"
150 ml. cyclohexanol	1	Glass	Calomel	Good	Slow	Poor	Very high	Inert	Good	
50 ml. methyl ethyl ketone + 25 ml. (CH <sub>3</sub> ) <sub>2</sub> CHOH	0.025	Glass	Calomel	Good	Good	Good	High	Reactive	Excellent	Unusually high potential rise
75 ml. methyl ethyl ketone + 75 ml. (CH <sub>3</sub> ) <sub>2</sub> CHOH	0.8	Glass	Calomel	Excellent	Very good	Good	High	Reactive	Very good	High final potential
75 ml. methyl ethyl ketone + 75 ml. (CH <sub>3</sub> ) <sub>2</sub> CHOH	0.8	Sb	Calomel	Excellent	Very good	Good	High	Reactive	Good	LiCl added, dwarfed
100 ml. methyl ethyl ketone	None	Glass	Calomel	Good	Slow	Very good	High	Reactive	Fair	Unusually high potential rise
100 ml. methyl ethyl ketone + 50 ml. (CH <sub>3</sub> ) <sub>2</sub> CHOH	5	Glass	Calomel	Excellent	Very good	Good	High	Reactive	Good	High final potential
75 ml. amyl ketone + 75 ml. (CH <sub>3</sub> ) <sub>2</sub> CHOH	0.3	Glass	Calomel	Good	Good	Good	High	Reactive	Good	
50 ml. anisole + 100 ml. (CH <sub>3</sub> ) <sub>2</sub> CHOH	1	Glass	Calomel	Good	Fair	Fair	Low	Inert	Good	
50 ml. anisole + 100 ml. (CH <sub>3</sub> ) <sub>2</sub> CHOH	1	Sb	Calomel	Good	Good	Good	Low	Inert	Poor	LiCl added, dwarfed
75 ml. isopropyl ether + 75 ml. (CH <sub>3</sub> ) <sub>2</sub> CHOH	0.8	Glass	Calomel	Good	Good	Fair	Low	Reactive	Excellent	Reactivity due to compounds
75 ml. isopropyl ether + 75 ml. (CH <sub>3</sub> ) <sub>2</sub> CHOH	0.8	Sb	Calomel	Good	Good	Good	Low	Reactive	Excellent	LiCl added
75 ml. dioxane + 75 ml. (CH <sub>3</sub> ) <sub>2</sub> CHOH	0.8	Glass	Calomel	Good	Slow	Fair	.....	.....	None	
95 ml. dioxane	5	Glass	Calomel	Good	Slow	Good	.....	.....	Very poor	Good inflection, strong acid
100 ml. benzyl alcohol	None	Glass	Calomel	Excellent	Very slow	Fair	Very high	Reactive	Very good	Medium viscosity



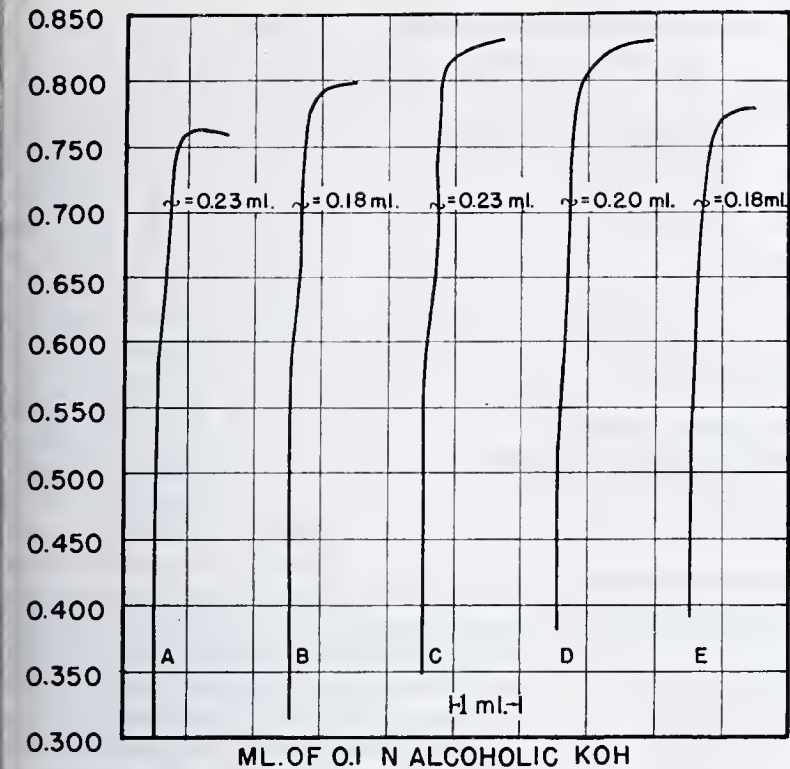


Figure 3. Optimum Water Content of Titration Solvent  
Free acid-base procedure. Potentiometric titrations of inherent acidity of solvent containing: A 0.05, B 0.5, C 2.5, D 5.0, and E 9.6% water. Titration cell. Glass electrode || calomel electrode. Titration solvent. 50 ml. of benzene, 50 ml. of isopropyl alcohol

pend upon the esters and acids present, and upon the materials associated with the esters. In the determination of the saponification number of castor oil in 10 grams of lubricating oil, the standard deviation is less than 0.05 ml. of 1.0 N acid, and the systematic error is less than  $\pm 1.96$  times the standard error. The accuracy, though not the precision, of both methods may be impaired by the nonreactivity of some acids or esters, and by the insolubility of some materials such as certain high molecular weight asphaltenes.

Experimental  
SOLVENT

**SELECTION OF TITRATION SOLVENT.** The first part of this investigation was concerned with the search for a solvent or solvent mixture to meet the specifications stated above. Exhaustive tests were made of solvents mentioned in the available literature, and of all others which might conceivably qualify. Those mixtures which evidenced possibilities were tested in various concentrations before abandonment. The characteristics of the major compositions tested are summarized in Table I; some of the concentration variations for individual mixtures are not listed, as they showed essentially the same characteristics as the parent mixture. The study resulted in finding two suitable media: (1) an approximately 50% mixture of benzene and isopropyl alcohol, and (2) an approximately 50% mixture of petroleum ether and isopropyl alcohol. The benzene-isopropyl alcohol was chosen because it has greater water tolerance and is a better solvent for asphalts and oxidized materials.

**OPTIMUM WATER CONCENTRATION OF TITRATION SOLVENT.** The presence of a certain amount of water in the benzene-isopropyl alcohol medium is beneficial, since it greatly reduces the resistance of the mixture and makes the measurement of potential difference between the electrodes considerably easier. A study was made of the optimum water content of the solvent for use in the free and combined acid methods already described.

**Titration of Free Acid or Base.** Mixtures of benzene and isopropyl alcohol were prepared containing 0.05, 0.5, 2.5, 5.0, and 9.6 (saturated) % water, and used as titration solvents in the following titrations:  
Blank titrations. One hundred milliliters of each solvent were titrated with 0.0852 N alcoholic potassium hydroxide.  
Hydrochloric acid-naphthone A titrations. Samples of 2 ml. of 0.1 N alcoholic hydrochloric acid and 2 ml. of 0.065 N alcoholic naphthone A (naphthenic acid) were dissolved in 100 ml. of each solvent-water mixture and titrated with 0.0852 N alcoholic potassium hydroxide.  
Zinc soap-oxidized oil titrations. A standard solution of zinc soap was prepared such that 4 ml. of solution contained 0.1 gram of zinc soap. Samples of 4 ml. of this solution were mixed with 5 grams of a typical oxidized motor oil, and titrated in each solvent-water mixture with 0.0852 N alcoholic potassium hydroxide.

The results of the titrations are summarized in Tables II and III, and the titration curves are shown in Figures 3 to 5. They indicate that while all solvent-water mixtures are suitable for the determination, the most desired titration characteristics are obtained using 0.5 to 2.5% water. On both sides of this range the titration curve is more dwarfed, and the characteristics of the titration are more erratic. The equilibration rate is more rapid as more and more water is added; however, the increase in rapidity is not pronounced after 0.5% water has been added. Balancing all factors such as equilibrium rate, the desire for a high water tolerance in the final medium, the desire for undwarfed titration curves, etc., the 0.5% value for water is considered optimum; it has been found very successful in routine application of the method.

**Titration of Combined Acids and Bases.** The same water-solvent mixtures used for the free acid-base investigation were employed for a study of the influence of water upon the combined acid-base determinations.

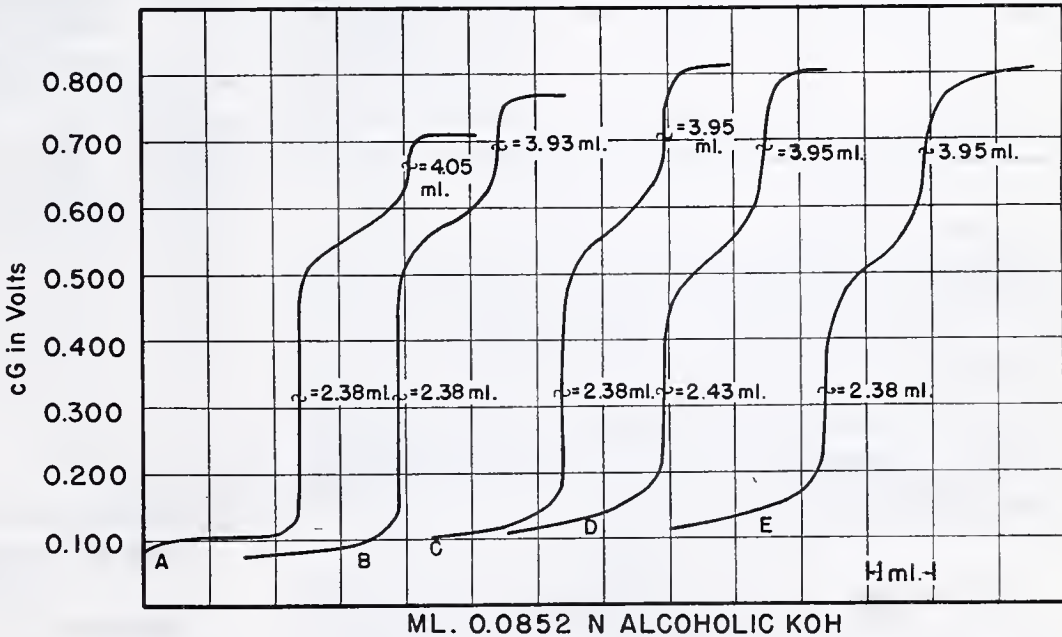


Figure 4. Optimum Water Content of Titration Solvent  
Free acid-base procedure. Potentiometric titrations of mixtures of hydrochloric acid and naphthone A in titration solvent containing: A 0.05, B 0.50, C 2.5, D 5.0, and E 9.6% water. Titration cell. Glass electrode || calomel electrode. Titration solvent. 50 ml. of benzene, 50 ml. of isopropyl alcohol



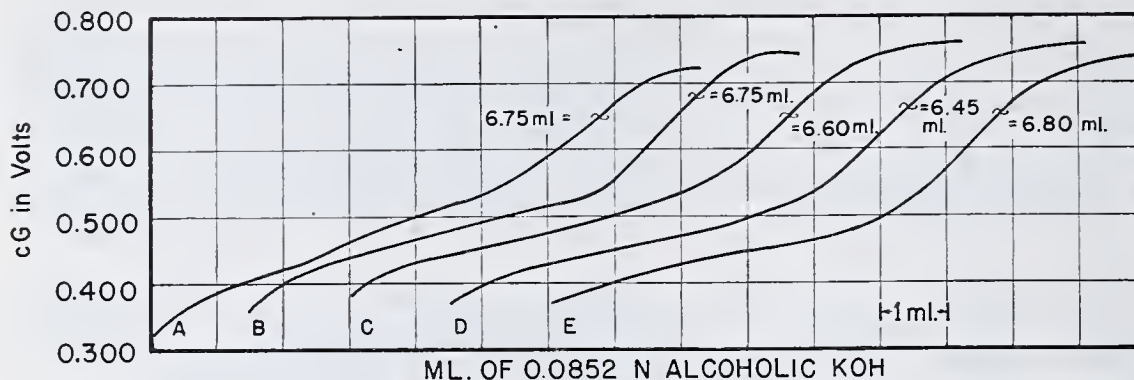


Figure 5. Optimum Water Content of Titration Solvent

Free acid-base procedure. Potentiometric titrations of 5 grams of oxidized oil and 0.100 gram of zinc soap in titration solvent containing: A 9.6, B 5.0, C 2.5, D 0.5, and E 0.05% water. Titration cell. Glass electrode || calomel electrode. Titration solvent. 50 ml. of benzene, 50 ml. of isopropyl alcohol.

Table II. Titration of Hydrochloric Acid and Naphthone A in Titration Solvent Containing Water

(Titration solvent: 50 ml. of benzene, 50 ml. of isopropyl alcohol)

Water Content of Titration Solvent % v.	0.0852 N Base					Remarks
	Solvent blank ML.	Strong Acids Found ML.	Theory ML.	Weak Acids Found ML.	Theory ML.	
0.05	0.23	2.38	2.38	1.44	1.41	Slow equilibrium, poor conductivity, potential irregular
0.5	0.18	2.38	2.38	1.37	1.41	Rapid equilibrium, stable potential
2.5	0.23	2.38	2.38	1.34	1.41	Very rapid equilibrium, very stable potential
5.0	0.20	2.43	2.38	1.32	1.41	Much like 0.5% water test
9.6	0.18	2.38	2.38	1.37	1.41	Much like water titration

Table III. Titration of Oxidized Oil Sample Containing Zinc Soap in Titration Solvents Containing Water

(Titration solvent: 50 ml. of benzene, 50 ml. of isopropyl alcohol)

Water Content of Titration Solvent % v.	Total Acid Number				Remarks
	Oil (5 Grams) plus Zinc Soap (0.1 Gram) At break	At cG = 0.65	Increase with Zinc Soap (0.1 Gram) At break	At cG = 0.65	
0.05	6.46	6.22	4.62	4.38	Slow equilibrium, unstable potential
0.5	6.50	6.04	4.66	4.20	Slow equilibrium, especially in break, unstable potential
2.5	6.46	6.08	4.62	4.24	Good equilibrium rate, slightly unstable potential
5.0	6.49	6.24	4.65	4.40	Rapid equilibrium, much like water titration
9.6	6.50	6.27	4.66	4.43	Like water titration

Samples of 10 grams of a typical oxidized oil were added to 100-ml. portions of the water-solvent mixtures, 10 ml. of 0.8 N alcoholic potassium hydroxide were added to each solution, and the resulting saponification mixture was refluxed gently for 2 hours. A blank was prepared for each solvent mixture by performing the identical steps, but omitting the sample. After saponification, blank and sample were removed from the hot plate, cooled, and transferred to titration beakers. The nonaqueous solution was titrated with 1 N alcoholic hydrochloric acid (the "first", or "nonaqueous" titration). One hundred milliliters of water were added to the reflux vessel, and the contents were boiled, cooled, and titrated with 1.0 N alcoholic hydrochloric acid (the "second" or "water" titration).

The data obtained are given in Table IV, and titration curves are shown in Figures 6 and 7. Two major effects of the presence of water in the saponification medium were noted: (1) The size of the second titration increases with increasing water concentration to a maximum at 2.5% water, then decreases slightly, and (2) when more than 0.5% water is present in the medium,

the salts formed during the nonaqueous titration tend to coat the electrodes and beaker walls, slowing the rate of equilibration and rendering the titrations almost impossible.

The second effect must naturally be avoided, so that a limitation of the water content to less than 0.5% is absolutely necessary. The first effect places a still greater limit on the water content. Previous to the determination of optimum water content, the possibility of determining combined acids by saponification in the benzene

isopropyl alcohol medium had been investigated. Several unsuccessful attempts were made to employ a single direct titration of the saponification mixture in the reflux vessel; in every case there was an unaccountable loss in caustic when the direct titration was employed, while no loss was noted when the double titration procedure was used. It was soon discovered by running many identical samples and blanks by both procedures, that the loss in caustic was proportional to the size of the second or water titration to be expected from the mixture. Apparently, the second titration results from the reaction of caustic with the reflux vessel with some other substance to form a residue that is insoluble in the titration solvent but

soluble in hot water. This material does not react with hydrochloric acid in the nonaqueous medium, but is readily titrated with acid in the aqueous medium. The analysis of typical water-soluble saponification residue is given in Table V. Nothing is known concerning the nature of these residues, but the presence of water seems to promote their formation. Therefore, if a direct titration is to be used, it is necessary to maintain

Table IV. Determination of Saponification Number of an Oxidized Oil in Titration Solvent Containing Water

(Titration solvent: 50 ml. of benzene, 50 ml. of isopropyl alcohol. Saponification conditions: approximately 10 grams of oil, 7 ml. of 1.0 N alcoholic KOH)

Water Content of Titration Solvent % v.	Saponification No. Mg. KOH/g.	Strong Acid Saponification No. Mg. KOH/g.	Remarks
0.05	4.(8)	1.(2)	Very rapid equilibration, negligible second titration, identical conditions
0.5	4.(0)	1.(0)	Rapid equilibration, second titration fairly small
2.5	3.(7)	0.(0)	Two phases on addition caustic. Equilibration slow, potentials unsteady, second titration high
5.0	6.(8)	1.(8)	Two phases on addition caustic, equilibration slow, second titration large
9.6	3.(7)	0.(8)	Two phases on addition caustic, equilibration rapid, fairly small second titration

Table V. Chemical Analysis of Typical Water-Soluble Saponification Residue

	Mg.
Total carbon	1642
CO <sub>2</sub>	29
B <sub>2</sub> O <sub>3</sub>	15
SiO <sub>2</sub>	100
Total potassium (K <sub>2</sub> O)	182
Total alkalinity (K <sub>2</sub> O)	182



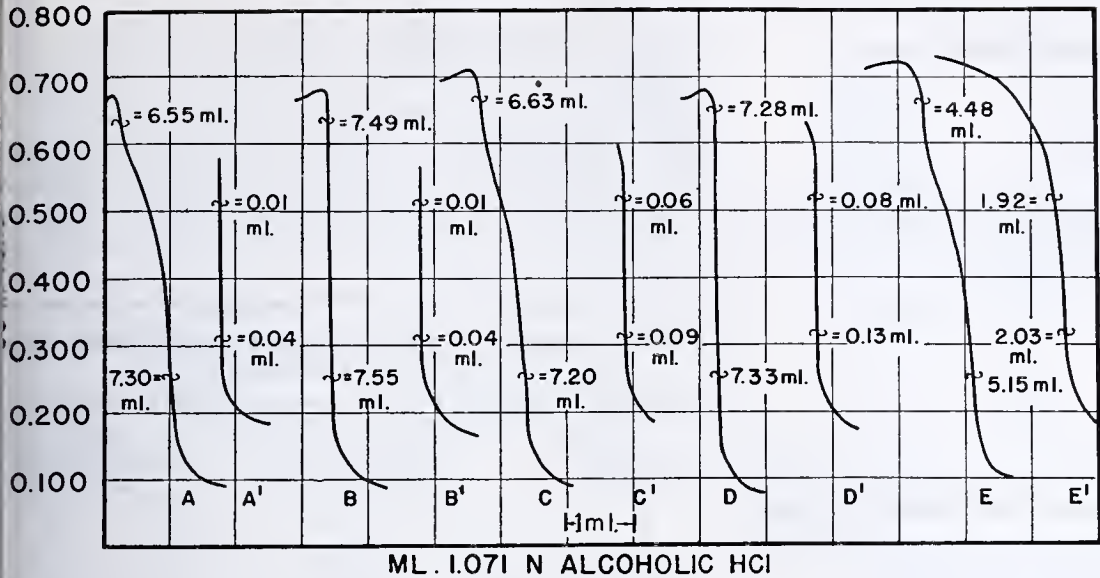


Figure 6. Optimum Water Content of Titration Solvent

Combined acid-base procedure. Potentiometric titration of aqueous and nonaqueous solutions resulting from saponification of indicated amounts of oxidized oil in presence of 50 ml. of benzene, 50 ml. of isopropyl alcohol, 10 ml. of approximately 0.7 N alcoholic potassium hydroxide, and indicated amounts of water; 2-hour reflux period

A. Nonaqueous solution, 11.71 grams of oil, 0.05% water	C'. Aqueous solution corresponding to C
A'. Aqueous solution corresponding to A	D. Nonaqueous solution, blank, 0.5% water
B. Nonaqueous solution, blank, 0.05% water	D'. Aqueous solution corresponding to D
B'. Aqueous solution corresponding to B	E. Nonaqueous solution, 10.31 grams of oil, 2.5% water
C. Nonaqueous solution, 10.18 grams of oil, 0.5% water	E'. Aqueous solution corresponding to E

low concentration of water in the saponification medium. difficulty is encountered if the water content is below 0.1%. NECESSARY PURITY OF TITRATION SOLVENT. In several thousand analyses of oxidized oils, it was found that the impurities present in ordinary commercial grades of benzene and isopropyl alcohol tend to produce erratic and inaccurate results when these solvents are used in the combined acid-base procedure. Deviations of as much as 30% have been obtained using the commercial solvents. This inaccuracy, not encountered in the case of free acid-base determinations, is apparently due to the presence of difficultly saponifiable compounds of sulfur or chlorine, or of materials which tend to form resins in the presence of excess caustic.

Commercial isopropyl alcohol occasionally contains appreciable amounts of unsaturated material, aldehydes, or ketones. On standing with excess potassium hydroxide, these materials form resins which color the alcohol yellow and cause objectionably high blanks in the combined acid-base procedure. The isopropyl alcohol used should develop no color on standing with solid potassium hydroxide, should contain less than 0.1% water by test, and should have low inherent acidity.

Commercial benzene generally contains saponifiable compounds of sulfur and chlorine which react more or less completely with potassium hydroxide. Since a great many factors can influence the rate of saponification of such compounds, considerable variation is obtained in the saponification numbers determined using

commercial benzene even when blank determinations are made. A typical analysis of ordinary commercial benzene is given in Table VI, and the analysis of a typical precipitate formed by reacting the benzene with potassium hydroxide in Table VII.

Ordinary commercial benzene or isopropyl alcohol can be easily purified to meet specifications by refluxing over potassium hydroxide for several hours, and then carefully fractionating.

ADDITION OF SOLUBLE ELECTROLYTE TO IMPROVE CONDUCTIVITY OF TITRATION SOLVENT. The addition of lithium chloride to the benzene-isopropyl alcohol medium was suggested as a means of reducing the resistance of the titration solvent. Trials were made using various concentrations of

lithium chloride in the medium, but it was found to react with caustic and cause a considerable error in the titration curve. To date, no soluble metallic salt has been found which is inert to potassium hydroxide in the medium. If such a salt can be found, its use may improve the characteristics of the solvent.

CHARACTERISTICS OF BENZENE-ISOPROPYL ALCOHOL TITRATION SOLVENT. *Equilibrium Rate.* All acids, bases, and salts, are ionized in benzene-isopropyl alcohol solvent to a degree comparable with that in water. Therefore it is assumed that the reactions taking place in the free acid-base titration are very rapid. As in aqueous solutions, saponification takes place at

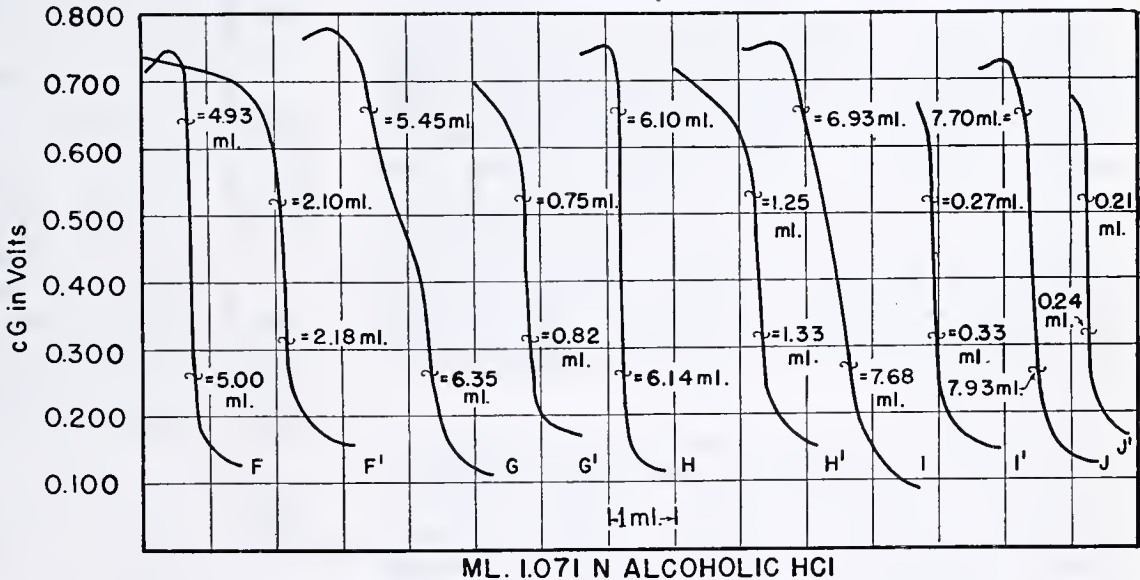


Figure 7. Optimum Water Content of Titration Solvent

Combined acid-base procedure. Potentiometric titration of aqueous and nonaqueous solutions resulting from saponification of indicated amounts of oxidized oil in presence of 50 ml. of benzene, 50 ml. of isopropyl alcohol, 10 ml. of approximately 0.7 N alcoholic potassium hydroxide and indicated amounts of water; 2-hour reflux period

F. Nonaqueous solution, blank, 2.5% water	F'. Aqueous solution corresponding to F
G. Nonaqueous solution, 10.13 grams of oil, 5.0% water	G'. Aqueous solution corresponding to G
H. Nonaqueous solution, blank, 5.0% water	H'. Aqueous solution corresponding to H
I. Nonaqueous solution, 8.15 grams of oil, 9.6% water	I'. Aqueous solution corresponding to I
J. Nonaqueous solution, blank, 9.6% water	J'. Aqueous solution corresponding to J

Titration cell. Glass electrode || calomel electrode



Table VI. Analysis of Commercial Grade Benzene

Purity by freezing point, %	99.92
Sulfur, %	0.0013
Chlorine, %	0.14
Carbonyl value, mole per 100 grams	0.006
Acetyl value, equivalent per 100 grams	0.002
Carbon, %	91.96
Hydrogen, %	7.79

Table VII. Analysis of Precipitate Formed during Saponification of Blanks Using Commercial Benzene

Sulfate ash, %	71.2
Potassium	Major constituent
Silicon, % <sup>a</sup>	1
Calcium, % <sup>a</sup>	0.1 - 1
Sodium, % <sup>a</sup>	0.1
Carbon, %	3.3
Hydrogen, %	1.3
Chlorine, %	23
Sulfur, %	0.09

<sup>a</sup> Order of magnitude only.

Table VIII. Influence of Time on Saponification Number of Castor Oil

(Saponification condition: 50 ml. of benzene, 50 ml. of isopropyl alcohol, 1.00 gram of castor oil, and 10 ml. of 0.904 N base)

Time of Reflux Min.	Saponification No. Mg. KOH/g.	Strong Acid Saponification No. Mg. KOH/g.	Saponified %
0	118	0.0	65
5	155	1.0	86
15	165	3.0	91
30	175	1.0	97
60	181	1.0	100
60 <sup>a</sup>	186	3.0	...
420 <sup>a</sup>	194	0.0	...

<sup>a</sup> Sample and blank contained 10 grams of SAE 30 lubricating oil.

slow reaction rates which must be taken into consideration in determining the time of reflux necessary for the saponification of combined acids and bases, and in estimating the necessary excess of caustic to saponify combined acids and bases in a reasonable length of time. Unlike titrations in aqueous media, titration in benzene-isopropyl alcohol solution requires that considerable time be allowed between increments of titrant to allow the electrode potential to come to reasonable equilibrium.

The course of events as each drop of titrant is added during a titration is indicated by the following observations.

(1) In many tests on plain oxidized oils there has been no evidence of the presence of a momentary excess of base immediately after adding an increment of the base solution. After addition of the base increment, the meter reading generally progresses steadily to a constant reading, in 1 to 6 minutes, and never tends to reach a maximum and decrease to a lower steady value on standing. (2) The meter reading progresses more quickly to an unchanging value at  $cG = 0.600$  to  $0.700$  volt than at  $cG = 0.400$  to  $0.500$  volt. (3) The increase of the base concentration of the solution takes place rather rapidly after the addition of a small increment of base titrant but the indicated  $cG$  potential reading follows slowly. Thus, it appears that time is required to replace the film of solution on the electrodes by a film of solution containing the added portion of the base. Rate of stirring has no appreciable influence on the time for equilibrations, provided it is adequate.

A great many variations in the manner of adding titrant have been tried—constant increments, constant waiting periods between additions of titrant, specification of rates of change of voltage to be accepted as indicating equilibrium, and many others. The present specification (1, 2) has grown out of a large amount of experience in making titrations in the benzene-isopropyl alcohol medium. It has been applied with success to a large number of standard samples in which the acid and basic strengths have varied from the highest to the lowest detectable by the method.

*Reaction of Caustic with Combined Acids and Bases in the Medium.* The excess of potassium hydroxide, and the time of reflux necessary for complete saponification of combined acids and bases, were experimentally determined using a sample of pure castor oil and a typical sample of oxidized oil. Tables VIII and IX summarize the results of saponifying equal amounts

of the oils for varying intervals of time in the presence of a large excess of potassium hydroxide. Table X gives the results of saponifying equal amounts of the oxidized oil in the presence of varying amounts of caustic, using a constant reflux time of 2 hours. A refluxing time of 2 hours and an excess of at least 20 ml. of 0.2 N potassium hydroxide apparently are satisfactory conditions, and allow sufficient analytical freedom. These conditions have been checked with a large number of known materials.

Table IX. Influence of Time of Reflux on Saponification Number of Oxidized Lubricating Oil

(Saponification mixture: 50 ml. of benzene, 50 ml. of isopropyl alcohol, 10 grams of oil, and 10 ml. of 0.904 N base)

Time of Reflux Hours	Saponification No. Mg. KOH/g.	Strong Acid Saponification No. Mg. KOH/g.
1	3.9	0.9
2	4.0	0.8
4	3.5	0.7
7.5	3.1	0.7
20	3.5	0.6

Table X. Influence of Excess Caustic upon Saponification Number of a Used Lubricating Oil

(Saponification condition: 50 ml. of benzene, 50 ml. of isopropyl alcohol, 10 grams of oil, and 2 hours' reflux)

0.904 N Alcoholic KOH in Medium Ml.	Saponification No. Mg. KOH/g.	Strong Acid Saponification No. Mg. KOH/g.
1.00	4.1	0.9
3.00	6.4	1.8
5.00	6.9	2.0
7.00	7.5	2.4
9.00	8.0	3.3
10.00	7.7	2.9

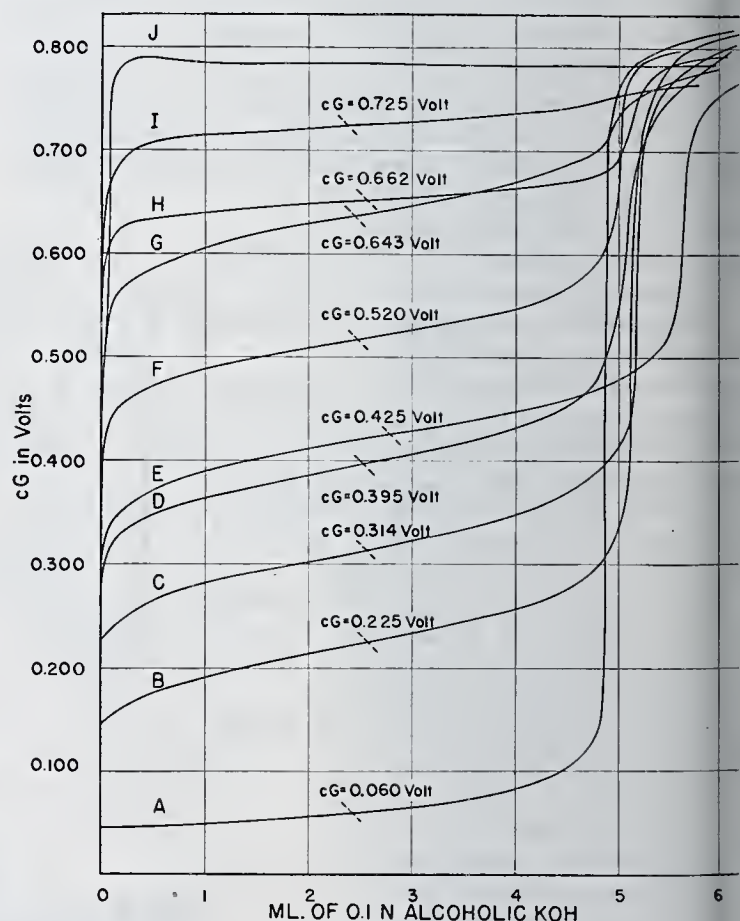


Figure 8. Influence of Acid Strength upon Free Acid Titration Curve

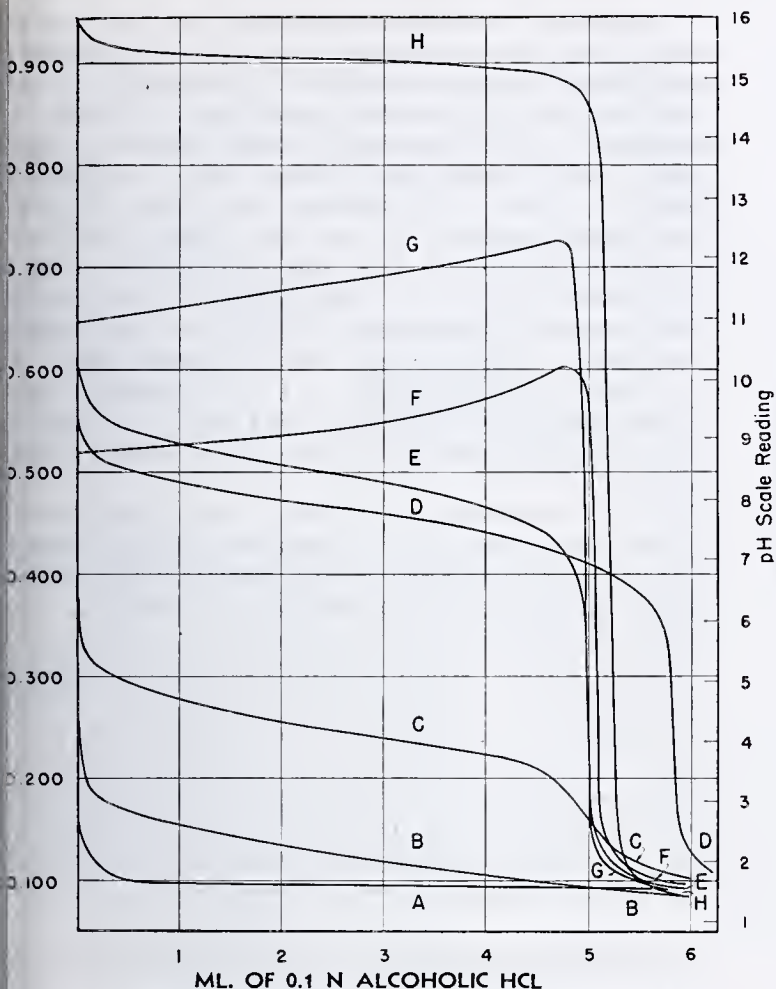
Potentiometric titration of acids with alcoholic potassium hydroxide:

- A. Hydrochloric acid
- B. Trichloroacetic acid
- C. Dichloroacetic acid
- D. Monochloroacetic acid
- E. Formic acid
- F. Acetic acid
- G. *p*-Nitrophenol
- H. *m*-Nitrophenol
- I. Phenol (hydroxybenzene)
- J. Blank

Titration cell. Glass electrode || calomel electrode

Titration solvent. 50 ml. of benzene, 50 ml. of isopropyl alcohol containing 1% water





**Figure 9. Influence of Base Strength upon Free Base Titration Curve**  
 Potentiometric titration of bases with alcoholic hydrochloric acid:  
 A. Blank  
 B. Pyridine  
 C. Hexamethylenetetramine  
 D. Ammonia  
 E. Butylamine  
 F. Sodium hydroxide  
 G. Potassium hydroxide  
 H. Tetraethylammonium hydroxide  
 Titration cell. Glass electrode || calomel electrode  
 Titration solvent. 50 ml. of benzene, 50 ml. of isopropyl alcohol containing 1% water

and found to produce complete saponification in practically all the trials. Certain compounds of phosphorus and a few other organic substances required more severe conditions. Throughout these tests, it was found that the benzene-isopropyl alcohol titration solvent is stable in the presence of excess base. It is not appreciably attacked even under conditions of basic saponification, such as 48 hours' reflux with excess strong base.

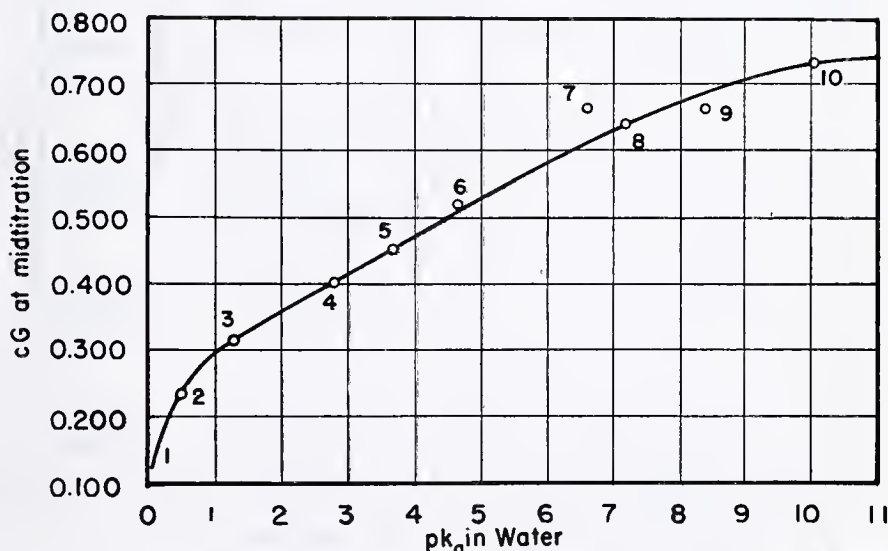
**Ionization of Acids and Bases in Benzene-Isopropyl Alcohol Titration Solvent.** While the apparent degree of ionization of most acids and bases in the benzene-isopropyl alcohol is measurably different from that in water, the relative acid or base strengths of the members of a series of acids or bases are in the same order in this titration solvent as in water; however, there is a better differentiation of the apparent degree of ionization of the more acidic materials in this titration solvent than in water. These relationships are illustrated by Figures 8 and 9, which give typical titration curves of various acids and bases titrated in the benzene-isopropyl alcohol titration solvent. Since it is assumed that the  $cG$  potential is a measure of the hydrogen activity of a aqueous solution, it is further assumed that the  $cG$  potential at the mid-titration indicates the relative acid or base strength of the substance in this titration solvent. That there is a definite relationship between this  $cG$  mid-titration point in benzene-isopropyl alcohol medium and the  $pK_a$  value in water is indicated by the smooth curve given in Figure

10, which is constructed by plotting these values for typical acids of varying strengths.

As the medium becomes less and less waterlike, strong acids tend to show considerably more irregularity than weak acids in their apparent degrees of ionization. An illustration of this is given by the family of titration curves in Figure 11. This series of curves was prepared by titrating identical mixtures of strong acids and weak acids in media of successively less waterlike properties. Evidently there is a large regular departure in the position of the buffering plateau in the case of the weak acid in contrast to an irregular change in that of the strong acid. There appears to be a tendency for the strong acid to show a maximum degree of ionization in solvents that are intermediate in waterlike or nonaqueous properties, while the weak acid shows a regular trend in this respect—that is, a weak acid shows a lower degree of ionization and a strong acid shows a somewhat higher degree of ionization in benzene-isopropyl alcohol medium than in water. Practically, this means that when using the benzene-isopropyl alcohol titration solvent there is a better chance of distinguishing between acids whose  $pK_a$  ( $H_2O$ ) are in the range of 1 to 3 than between those whose  $pK_a$  ( $H_2O$ ) range from 6 to 8.

**Titration Curves for Oxidized Oils.** The acidity or basicity present in oxidized oils is by no means necessarily due to a single acid or base. It may be and probably is made up of a large number of different components, each having its own individual degree of ionization. As a result, in contrast to the clear titration curves produced by single acids or bases (Figures 8 and 9), the oxidized oils yield indefinite titration curves of the type illustrated by curves *B* and *E* of Figure 1 and *B* of Figure 2. In most cases there is an inflection on the curve to indicate the end point; however, in some instances, the titration curves exhibit no recognizable inflection. In such cases, in order to measure the changes of acidity or basicity taking place during the oxidation of the oil, it is necessary to establish definite  $cG$  reference points to be used to indicate the end

point of the titration in much the same way that indicators are used for this purpose. For the purposes of the methods presented in this paper, the points  $cG = 0.650$  volt for total acid and  $cG = 0.236$  volt for total base were chosen to represent the end point indicating the transition to strong base and strong acid, respectively. These points were selected because for all



**Figure 10. Relation of Acid Strength in Water to Acid Strength in Benzene-Isopropyl Alcohol Titration Solvent**

- |                          |                          |
|--------------------------|--------------------------|
| 1. Hydrochloric acid     | 6. Acetic acid           |
| 2. Trichloroacetic acid  | 7. Thiophenol            |
| 3. Dichloroacetic acid   | 8. <i>p</i> -Nitrophenol |
| 4. Monochloroacetic acid | 9. <i>m</i> -Nitrophenol |
| 5. Formic acid           | 10. Phenol               |

Based on titration curves shown in Figure 8



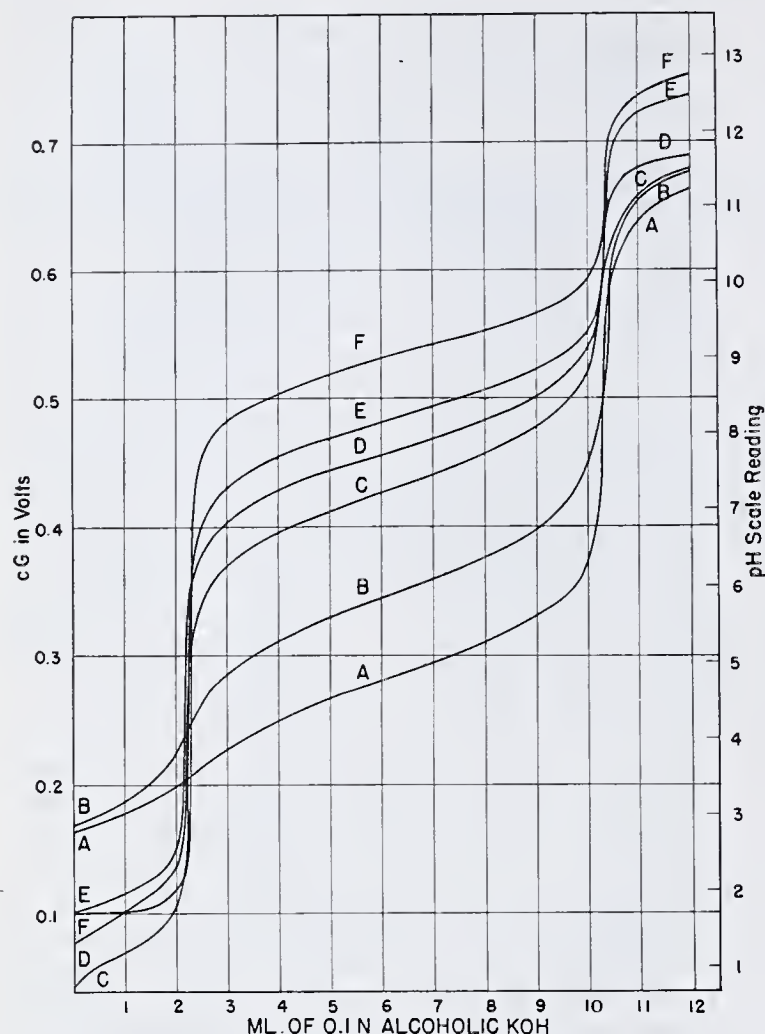


Figure 11. Effects of Nonaqueous Medium Characteristics upon Free Acid Titration Curves

Potentiometric titration of hydrochloric and acetic acids with alcoholic potassium hydroxide in the following media:

- A. 125 ml. of water  
 B. 62 ml. of methanol, 62 ml. of water  
 C. 125 ml. of methanol (0.5% water)  
 D. 125 ml. of ethanol (0.5% water)  
 E. 125 ml. of isopropyl alcohol (0.5% water)  
 F. 62 ml. of benzene, 62 ml. of isopropyl alcohol containing 1% water  
 Titration cell. Glass electrode || calomel electrode

samples that give good inflections in the titration curves, the inflections occurred at or near these points. The point  $cG = 0.650$  volt is also near the point at which phenolphthalein changes color in isopropyl alcohol-benzene solution.

In the earlier work on the method, end points were chosen at approximately  $cG = 0.620$  volt; however, in an effort to get better correlation with the higher results usually obtained by the available colorimetric indicator methods, it was decided to fix the arbitrary end point at  $cG = 0.650$  volt, and this value was incorporated in the method. Although  $cG = 0.650$  volt may generally be reached by proper handling of the apparatus and use of a correct amount of sample, a value of  $cG = 0.620$  volt, or even  $cG = 0.600$  volt, could be fixed upon for cooperative work if desired. Of course, if a definite "break" of more than 0.025 volt by 0.1 ml. of 0.1  $N$  base is realized in this range, the end point is logically chosen at the inflection in the titration curve. Many attempts were made to choose the insignificant break or "dip" occurring between  $cG = 0.600$  and 0.650 volt, which is often found with oxidized oils, but precision was generally poor and erratic and this practice was abandoned.

**Solvent Action.** The benzene-isopropyl alcohol titration solvent is a very good solvent for practically all petro-

leum products. It dissolves or disperses most petroleum products to form a solution which tolerates several milliliters of water without forming a second phase. In addition, it dissolves most resins and organic polymers, alkali soaps, and esters. It is not a good solvent for inorganic salts, salts of low molecular weight organic acids, or organic salts of heavy metals; such salts precipitate in a more or less flocculent form. This is a decided disadvantage where more than one acid hydrogen is to be found in the titrated acid. In such cases, neutralization of the first hydrogen precipitates the acid salt from solution, and successive hydrogens must be neutralized by titrating the solid phase first precipitated, converting it into a second insoluble phase. Thus the titration of polybasic acids is not very satisfactory as shown by the typical titration curves given in Figure 12; however, very few such acids are ordinarily found in new or oxidized petroleum products.

**Acidic Characteristics of Metallic Salts in the Medium**  
 Metallic salts, although for the most part insoluble in benzene-isopropyl alcohol, react more or less readily with potassium hydroxide during the titration of free acids, or during the saponification of combined acids and bases, to form the respective hydroxide, and they generally yield reproducible titration curves (see Figure 13). In general, since the salts and the hydroxide of the metals are insoluble in the medium, the reaction of the salts with caustic and the reaction of acid with the hydroxide formed are very slow and give rise to slow equilibration of the electrode potentials and erratic points on the titration curve. The erratic potential readings are obtained because the potential change by a very large amount as a result of the momentary e-

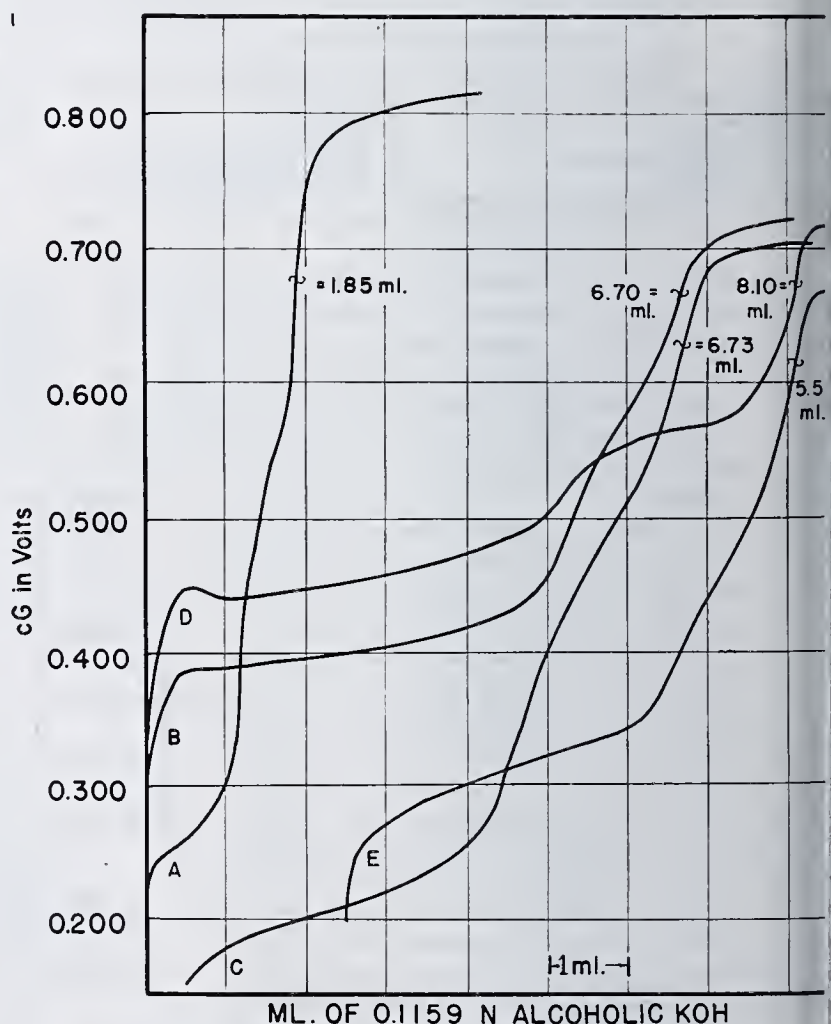
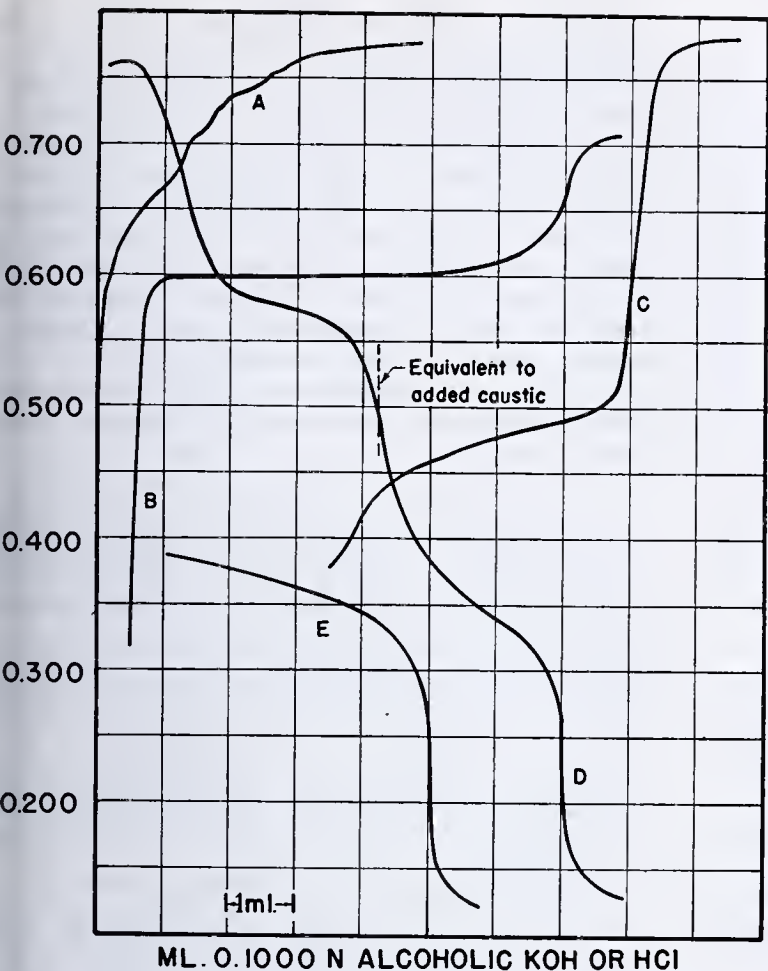


Figure 12. Titration of Polybasic Acids

Potentiometric titration of polybasic acids with alcoholic potassium hydroxide:  
 A. 2.0 ml. of 0.1907  $N$  aqueous phosphoric acid. Theoretical total titration, 3.29 ml.  
 B. 0.055 gram of succinic acid. Theoretical total titration, 8.20 ml.  
 C. 0.054 gram of maleic acid. Theoretical total titration, 8.20 ml.  
 D. 0.094 gram of sebacic acid. Theoretical total titration, 8.15 ml.  
 E. 0.077 gram of *o*-phthalic acid. Theoretical total titration, 8.15 ml.  
 Titration cell. Glass electrode || calomel electrode  
 Titration solvent. 50 ml. of benzene, 50 ml. of isopropyl alcohol containing 1% water





ML. OF 0.1000 N ALCOHOLIC KOH OR HCl

Figure 13. Titration of Metallic Salts

potentiometric titration with alcoholic potassium hydroxide:  
 A. 0.1172 gram of copper palmitate  
 B. 0.0276 gram of lithium chloride  
 C. 0.1170 gram of zinc soap  
 potentiometric titration with alcoholic hydrochloric acid:  
 D. 0.10 gram of calcium soap, saponified with 40 ml. of 0.2 N alcoholic potassium hydroxide (combined acid-base procedure). E. 0.1000 gram of zinc soap  
 titration cell. Glass electrode || calomel electrode  
 titration solvent. 50 ml. of benzene, 50 ml. of isopropyl alcohol containing 1% water

## APPARATUS

Although most glass electrode and meter assemblies intended for pH determination function satisfactorily in an aqueous medium, many are not directly applicable for similar measurements in a nonaqueous medium. Although not all the exact specification limits have been established, satisfactory results are obtainable by the proposed method when the electrodes and meter possess certain essential characteristics which are noted below.

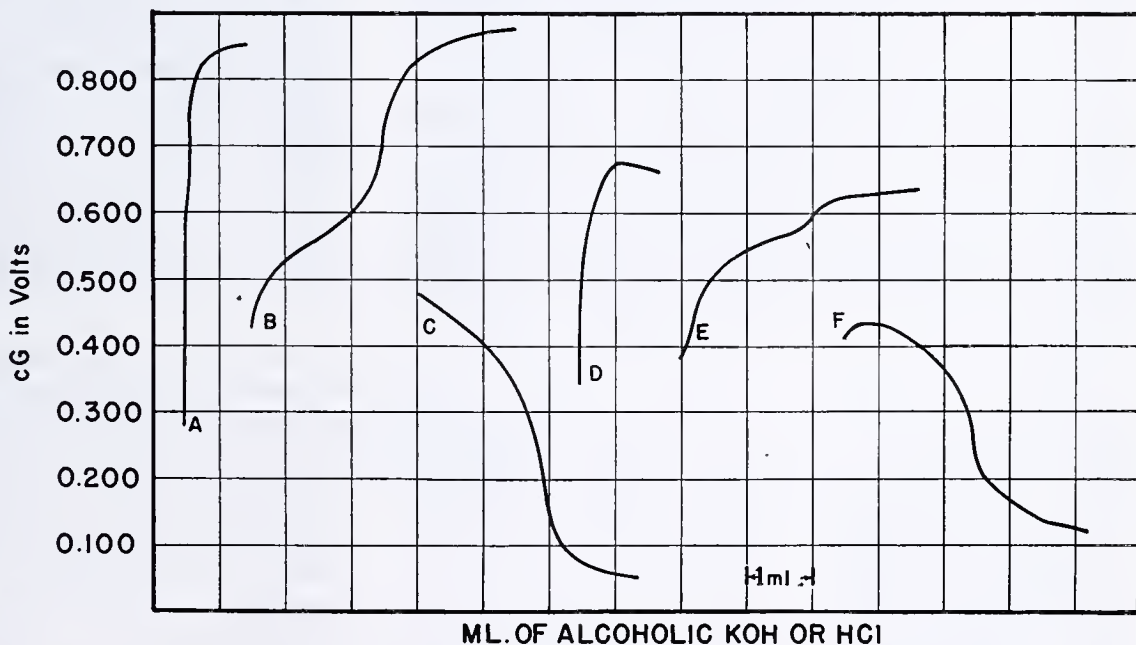
**ELECTRODES. Selection of Electrode System.** Most of the electrodes suggested in the literature were experimentally evaluated for the measurement of hydrogen-ion activity and for use as reference electrodes. The principal electrodes tried are listed above; their faults were verified by experiment.

A considerable number of reference electrodes were tried, most of them differing only in the type of salt bridge. Many variations of the salt bridge were applied, including the use of various organic electrolyte media. The best all-around reference electrode was found to be a saturated calomel (aqueous) half-cell with a ground-glass contact. Of several reference electrodes tried, the only one that gave suitable service was the Beckman No. 4970A calomel electrode having a ground-glass sleeve. This is a pencil-type electrode having a permanent inner cell surrounded by potassium chloride solution. Contact between the electrolyte solution and titration medium is made by a removable ground-glass sleeve.

The only electrodes which were found suitable for use as hydrogen-ion indicating electrodes in nonaqueous media were the thin (low-resistance) glass electrode and the modern sturdy (high-resistance) glass electrode; the former was discarded because of its extreme fragility. All-glass electrodes of the sturdy type available from American manufacturers were tested for use in the benzene-isopropyl alcohol titration solvent. The only ones found suitable were the Beckman No. 4990 and No. 4990E electrodes. All others (including the Beckman No. 4990X electrode) failed in some respect to give satisfactory results or service. The No. 4990 electrode is 12.5 cm. (5 inches) long with a diameter of 1.445 cm. (0.578 inch) and a bulb of Corning 015

ss produced by each increment of titrant, and then fade slowly back to the normal potential as the excess is slowly reduced by the reaction. This behavior not only causes error in estimation of the free or combined organic acids, but also prolongs the titration to an impractical limit.

In an attempt to overcome metal salt interference, sodium oxalate was added to the medium before the titration, in the hope that the sodium oxalate would precipitate the heavy metals as oxalates, leaving the non-interfering sodium salts in their free state. However, use of the oxalate did not reduce the interference by the metal salts and in some cases a considerably greater error resulted from its use. No attempt was made to find a more suitable anion than oxalate ion.



ML. OF ALCOHOLIC KOH OR HCl

Figure 14. Effect of Improper Maintenance of Electrodes upon Free Acid-Base Titration Curves

A. Titration for free acid in blank. Electrodes carefully prepared according to directions given in method  
 B. Titration for free acid in 4.99 grams of A.S.T.M. cooperative test sample N-8. Electrodes properly prepared according to method  
 C. Titration for free base in 5.06 grams of A.S.T.M. cooperative test sample N-4. Electrodes properly prepared according to method  
 D. Titration for free acid in blank. Electrodes treated in recommended manner after making titration C, but not allowed to soak 2 minutes in distilled water before titrating  
 E. Titration for free acid in 5.00 grams of A.S.T.M. cooperative test sample N-8. Electrodes treated in recommended manner, but not allowed to soak 2 minutes in distilled water before titrating  
 F. Titration for free base in 5.04 grams of A.S.T.M. sample N-4. Electrodes treated in recommended manner, but not allowed to soak 2 minutes in distilled water before titrating  
 titration cell. Glass electrode || calomel electrode  
 titration solvent. 50 ml. of benzene, 50 ml. of isopropyl alcohol containing 1% water



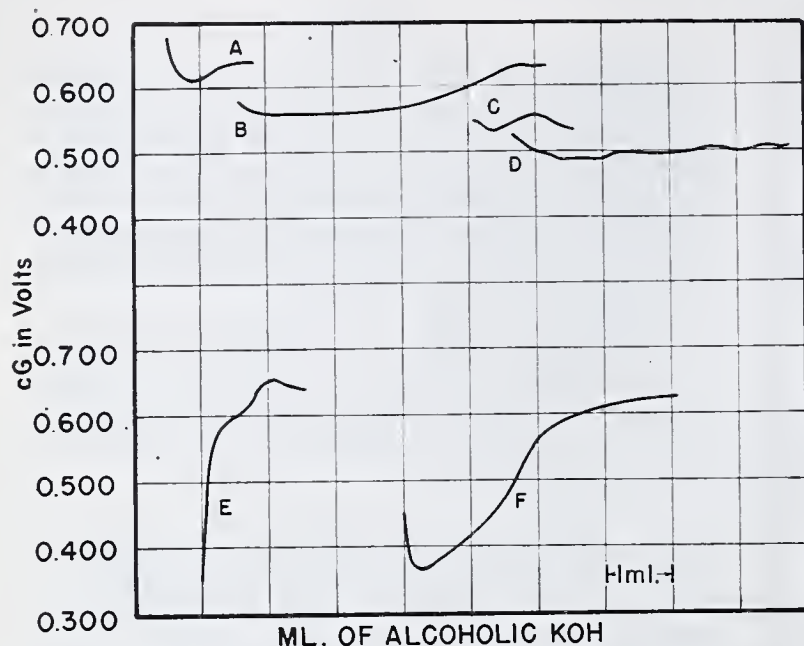


Figure 15. Effect of Improper Maintenance of Electrodes upon Free Acid-Base Titration Curves

- A. Titration of free acid in blank. Electrodes, following nonaqueous titration, washed down with benzene-isopropyl alcohol only, calomel electrode sleeve not removed before titrating.
- B. Titration of free acid in 5.05 grams of A.S.T.M. cooperative test sample N-7. Electrodes prepared as in A.
- C. Titration of free acid in blank. Electrodes, following nonaqueous titration, washed down with benzene-isopropyl alcohol only, and wiped dry. Sleeve of calomel electrode removed, wiped dry, and replaced in recommended manner.
- D. Titration of free acid in 5.02 grams of A.S.T.M. cooperative test sample N-5. Electrodes prepared as in C.
- E. Titration of free acid in blank. Electrodes, following nonaqueous titration, rinsed with isopropyl alcohol-benzene, then with water. Immersed in water, for 0.5 minute, then dried. Calomel electrode sleeve not removed prior to titration.
- F. Titration of free acid in 5.00 grams of A.S.T.M. cooperative test sample N-5. Electrodes prepared as in E.
- Titration cell. Glass electrode || calomel electrode  
Titration solvent. 50 ml. of benzene, 50 ml. of isopropyl alcohol containing 1% water

glass; it has a resistance of 200 to 300 megohms at room temperature. This type of electrode has been used with good success in the analysis of over 5000 samples of oxidized oils over 3 years. The No. 4990E electrode, which is especially designed for use in the high pH range, has given satisfactory service for several months; however, it has not been tested to the extent that the No. 4990 electrode has.

Although the thin, low-resistance glass electrodes that do not require electrical shielding function reasonably well, they do not have the chemical or physical durability required for continued use in nonaqueous media.

The expected life of the electrodes is 3 months or more of continuous use 8 hours per day in nonaqueous media. The period of satisfactory performance is considerably prolonged by keeping the electrodes immersed in distilled water between titrations. Thus, upon completion of a titration it is desirable to remove the nonaqueous titrated solution and replace it with water.

**Preparation and Testing of Electrode System.** Consistently reproducible results may be obtained by different operators, provided they are trained to prepare the electrodes properly for use prior to each determination. Improper preparation of the electrode system has generally shown the following manifestations in the blank titration on the titration solvent: (1) production of a titration curve that fails to exceed  $cG = 0.700$  volt upon addition of 0.5 to 1.0 ml. of standard base, (2) extremely slow rate of equilibration between increments of base, (3) little or no change in potentials with the initial increments of base, and (4) tendency for the titration curve to begin at too high  $cG$  potentials. In addition, improper electrode preparation is indicated during the titration of a sample whenever the initial increments of base cause the  $cG$  potentials to decrease instead of increase. Although the glass electrode may be partially at fault, this improper performance is usually caused by the condition of the interface between the salt solution in the calomel electrode and the titration solvent.

A defective or faulty glass electrode is indicated when the electrode system gives lower than normal potentials in aqueous solutions at pH 11 to 12 and when the replacement of the glass electrode with a new one increases the maximum  $cG$  potential attainable in the blank and/or increases the rate of equilibration between increments of base. Such a defective condition is not generally detectable by visual examination. The reference electrode is rarely defective when new; it is more likely that it is apparently defective because of improper preparation for use or an unsuccessful attempt to repair or replace a broken part, such as a sleeve.

The following preparatory steps must be taken prior to each titration to secure proper performance in nonaqueous titration.

1. Rinse the electrodes with benzene-isopropyl alcohol titration solvent, then with water, and wipe thoroughly with a clean cloth. Remove the ground-glass sleeve from the calomel electrode and wipe both ground surfaces of the sleeve with a clean cloth. Replace the sleeve and allow potassium chloride electrolyte to fill the ground area.

If this treatment does not leave the electrodes thoroughly clean, immerse them in chromic acid cleaning solution for several minutes, rinse them thoroughly with distilled water, and allow them to stand in distilled water for several hours. If required, use cleaning solution to clean the ground surfaces. Whenever cleaning solution is used to clean the calomel electrode, drain the remaining electrolyte from the electrode and replace with fresh electrolyte solution.

2. Immerse both (clean) electrodes in distilled water for at least 2 minutes, remove from the water, and wipe with a clean cloth as before. Again remove the sleeve from the calomel electrode, dry each ground surface with a clean cloth, and replace the sleeve in such a manner that the ground surfaces are completely and copiously wetted with electrolyte solution. Be certain to leave a ring of electrolyte solution in the capillary space above and below the sleeve, so that good electrical contact is established at the electrolyte-titration solvent interface.

3. At all times, maintain the level of the electrolyte solution in the calomel electrode above the point to which the electrode is immersed in the titration solvent or other medium.

4. Immerse the electrodes in the solution to be titrated and proceed with the titration.

To test a new set of electrodes, or an old set suspected of deterioration, make a blank titration of the benzene-isopropyl alcohol titration solvent as directed (1). Record the time required for equilibration after each increment as well as the final potential produced. Continue the titration, using 0.05-ml. increments, until the potential reaches its maximum value. When the electrode system is in good order and properly prepared, this test should yield a maximum potential on the blank titration curve of  $cG = 0.7$  to  $0.8$  volt and the average equilibration time to produce an unchanging potential should be less than 5 minutes per 0.05-ml. increment of base added.

In order to demonstrate further the importance of the preparatory steps in securing satisfactory performance of the electrode system, experimental titrations were made after omitting the essential preparatory steps one at a time. The conditions of the test and the resulting titration curves are given in Figures 14 and 15. For comparison, some actual titration curves obtained by typical inexperienced operators are given in Figure 16.

These tests clearly indicate the importance of removing the sleeve from the calomel electrode and immersing both electrodes in water prior to every titration in benzene-isopropyl alcohol titration solvent. The elimination of these steps causes serious distortion of the titration curve. Apparently a water film adheres to the surface of the glass electrode for the duration of the titration and as long as this film is unbroken it provides the necessary conditions for equilibrium. Prolonged contact with the nonaqueous solvent (more than 90 minutes) tends to deactivate the electrode system, possibly by removing the water at points of contact. However, this does no permanent harm because contact with water during the preparation for the next



titration restores the activity. The contact with water also removes any caustic film remaining on the electrodes from a previous determination.

**Effects of Continued Use on Characteristics of Electrode System.** With continued use in nonaqueous solutions, the characteristics of the glass electrodes change in such a manner that the maximum potential, which can be obtained in the presence of excess base, steadily drops. Thus, after 6 months' continued use, the maximum potential obtainable may be only equivalent to a calculated  $cG$  value of 0.600 volt. This electrode deterioration is in accord with the corroborating evidence found by others (10). The continued use of glass electrodes in a nonaqueous titration solvent alters their characteristics in two ways: They tend to exhibit a gradually increasing reluctance to follow changes in hydrogen-ion activity in a normal way, and they become less and less accurate in measuring the hydrogen-ion activity.

When a new glass electrode is put into use, it equilibrates very rapidly for the first few weeks, and then less and less rapidly. Eventually it becomes so slow as to be unsatisfactory for nonaqueous titrations and must be discarded. Thus it is desirable to have a test of equilibrium rate for electrodes which will indicate their usefulness. Such a test is included in the performance test of electrodes in the procedures for free and combined acid and base (1, 2).

All glass electrodes show a certain amount of error in measuring the pH of aqueous solutions or the  $cG$  of nonaqueous solutions, especially in alkaline solutions. This error is gradually increased by continued use in nonaqueous medium until the electrodes become impractical for further use (Table XI). It is because of such errors in measurements by the glass electrode that no two electrode systems will give identical  $cG$  readings for a given solution of partially neutralized acids or bases in nonaqueous solution. Thus, some means must be used to apply a correction to the electrodes in order to determine accurately the important reference potentials,  $cG = 0.236$  and  $0.650$  volt. This correction is determined by testing with standard nonaqueous buffers (2) whose  $cG$  readings on the basis of tests with ideal electrodes are assumed to be standard for  $cG = 0.236$  and  $0.650$  volt. When the errors become so great that the electrodes will not reach the calculated value for  $cG = 0.650$  volt in the presence of a slight excess of strong base, they must be discarded; in fact, for best results the electrodes should be discarded if they will not give a higher reading than  $cG = 0.700$  volt.

The continued use of the calomel electrode in nonaqueous

Table XI. Comparison of New and Used Glass Electrodes

(Calomel electrode used as reference)

Test	New Glass Electrode	Average Glass Electrode	Borderline Glass Electrode	Unsatisfactory Glass Electrode
pH reading in pH 4.00 buffer	4.00	4.00	4.00	4.00
pH reading in pH 7.00 buffer	7.02	7.03	7.05	7.05
pH reading in pH 10.00 buffer	9.95	9.79	9.28	8.83
pH reading in pH 11.00 buffer	10.76	10.53	10.03	9.37
Maximum $cG$ reading reached in free acid number blank, volt	0.796	0.742	0.720	0.692
Time required to attain maximum potential when immersed in benzene-isopropyl alcohol containing excess caustic, min.	1	2.5	7	15
$cG$ reading in $cG = 0.650$ volt nonaqueous buffer solution, volt	0.649	0.629	0.617	0.608

solutions has no apparent effect upon its useful characteristics. If proper care is exercised in its preparation and maintenance, it should last indefinitely.

**TITRATION METER.** Several commercial electronic voltmeters were tested for applicability to the measurement of the voltages between the glass-calomel electrodes in the isopropyl alcohol-benzene medium.

The most satisfactory meters were the Beckman Model M (or Model O) pH meters manufactured by the National Technical Laboratories, the electronic voltmeter of equivalent characteristics (26), and the dual alternating current titrometer described by Penther and Rolfson (25). Except for the Beckman meters and electrodes, none of the commercial apparatus tested was applicable without modification to titrations in the benzene-isopropyl alcohol titration solvent, although all were applicable to titrations in an aqueous medium. In some cases, substitution of the Beckman calomel electrode for the reference electrode, furnished by the manufacturer, converted an unsatisfactory apparatus to an apparatus useful for titration in nonaqueous media.

In general terms, a suitable meter should meet the following specifications:

The meter must be a voltmeter or potentiometer that will operate with an accuracy of  $\pm 0.005$  volt and a sensitivity of  $\pm 0.002$  volt, over a range of at least  $\pm 0.5$  volt, when used with

an electrode system having 500 megohms' resistance and when the resistance between the electrodes falls within the range of 0.2 to 20 megohms. The meter must be protected from stray electrostatic fields, so that when it is connected to the electrode system no permanent change in meter readings, over the entire range, is produced by touching with a grounded lead any part of the exposed surface of the glass electrode, the glass electrode lead, the titration stand, or the meter. A satisfactory meter should be designed to operate on an input of less than  $5 \times 10^{-12}$  ampere when an electrode system of 1000 megohms' resistance is connected to the terminals and should be provided with a satisfactory terminal (26) to connect the shielded connection wire from the glass electrode to the meter without interference from the presence of external electrostatic fields.

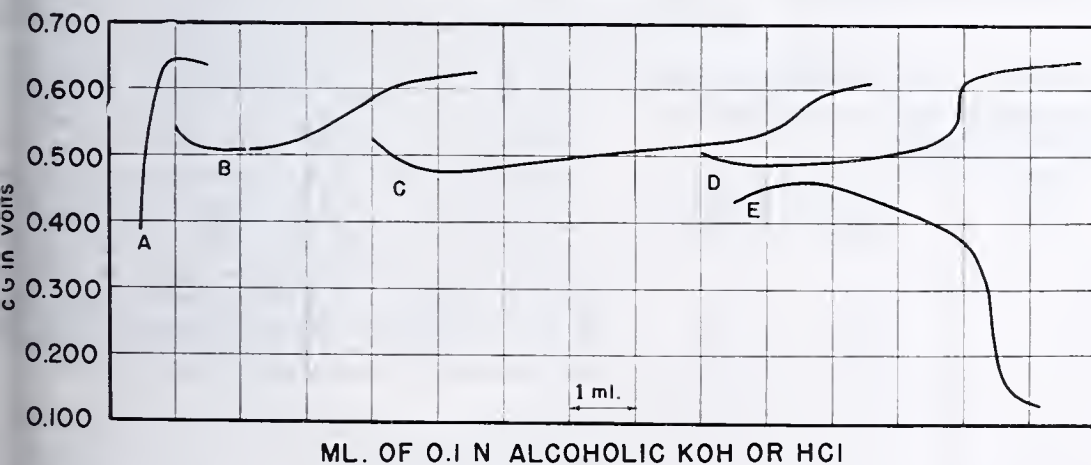


Figure 16. Free Acid-Base Titration Curves Obtained by Typical Inexperienced Operator

Potentiometric determinations of free acid by titration with alcoholic potassium hydroxide:

- A. Blank
- B. 10 grams of A.S.T.M. cooperative sample N-5
- C. 10 grams of A.S.T.M. cooperative sample N-7
- D. 10 grams of A.S.T.M. cooperative sample N-8

Potentiometric determination of free base by titration with alcoholic hydrochloric acid

- E. 10 grams of A.S.T.M. cooperative sample N-4

Titration cell. Glass electrode || calomel electrode

Titration solvent. 50 ml. of benzene, 50 ml. of isopropyl alcohol containing 1% water



**TITRATION STAND.** For most effective application of the methods given in this paper, it is necessary to have some convenient manner of mounting the electrodes, stirrer, burets, and titration beakers in a compact unit, so that cleaning, adjusting, and replacing solutions can be done with a minimum of effort and the titrations can be made conveniently and rapidly. A stand (1, 2) was developed especially for this application and has been found very efficient. It is now available from a commercial supply house.

**SAPONIFICATION APPARATUS.** The greater part of the investigation of the combined acid-base determination was done using the conventional type of reflux apparatus with Erlenmeyer flask and Allihn condenser. When the properties of the solvent became better understood and the single titration procedure became possible, a special saponification reflux apparatus was devised for carrying out the saponification directly in the titration beaker (2); this apparatus was constructed by fitting the titration beaker with an immersion-type condenser.

## Discussion

During this investigation many attempts were made to apply indicator methods to the determination of acidity in dark petroleum products for the very practical reasons of economy in time and equipment. All indicators and mixed indicators recommended in the available literature were tested, but were rejected, because the color change could not be seen in dark samples, took place too early or too late as compared with potentiometric titration results, or was gradual or indistinct. Spotlights, ultraviolet lights to induce fluorescence, white porcelain spatulas pressed against the sides of the titration flask, and tilting the flask were tried to obtain better observation of the end point, but without success.

No usable relationship has been found between acid numbers of dark oils obtained by the potentiometric method, and those obtained by the usual colorimetric methods. This is illustrated in Table XII, which shows the acid numbers obtained for several typical series of oxidized oils chosen at random from the results of a large number of oil oxidation tests. For combined acid (saponification) number, the agreement between the results of colorimetric and potentiometric methods is somewhat better (Table XIII).

Early in the development of the methods for free acid and free base, it became apparent that the slow addition of titrant could cause a gradual saponification of easily saponified materials during the titration.

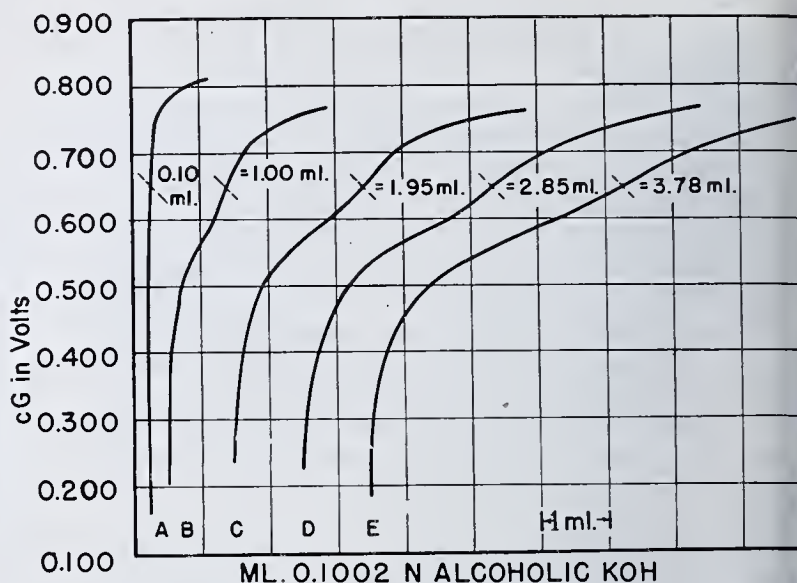


Figure 17. Influence of Sample Size upon Determination of Free Acid

Potentiometric titration of following amounts of oxidized oil with alcoholic potassium hydroxide: A 0.00, B 2.00, C 4.10, D 6.11, E 8.18 grams  
Titration cell. Glass electrode || calomel electrode  
Titration solvent. 50 ml. of benzene, 50 ml. of isopropyl alcohol containing 1% water

In order to determine the extent of the errors due to the presence of easily saponifiable material, a standard solution of oxidized oil and castor oil in benzene-isopropyl alcohol titration solvent was employed. This was prepared so that 100 ml. of solution contained 5 grams of oxidized oil and 3 grams of castor oil. Portions (100 ml.) of this solution were titrated to successive higher e.m.f. values with alkali, and were immediately back-titrated with alcoholic hydrochloric acid to see if the curve would retrace themselves—i.e., to determine whether or not saponification had taken place. It was found that the back-titration with acid retraces the base titration curve within the practical limits of experimental error when the titration stopped at any point below  $cG = 0.650$  volt. If the titration stopped at  $cG = 0.709$  volt, a small amount of saponification takes place. Thus, if the end point in the free acid-titration occurs near  $cG = 0.650$  volt, it can be safely assumed that no saponification has taken place unless the sample contains ester more readily saponified than castor oil. If the end point occurs above  $cG = 0.700$  volt, further evidence that some saponification has not occurred is necessary.

The optimum sample size has been found by experience from the results obtained in a great number of analyses. The main factors governing the choice of sample size were found to be:

1. Amount of precipitated salts formed during titration. This factor applies to free acid and free base determinations only since the amount of caustic added in the saponification procedure controls the amount of salts formed during the titration in the case of combined acid-base determinations. When too much of the flocculent precipitate of salts is formed in the titration vessel, it interferes by occluding some sample. When this happens, the equilibration is slow and the titration curve is abnormal, both conditions producing considerable error.
2. Characteristics of the break. The sharpness of the breaks in the titration curves for oxidized oils depends to a considerable extent upon magnitude of the titration and, for that reason, on the size of sample used. Examples of this dependence are shown for free acid determinations in Figure 17, and for combined acid-base determinations in Figure 18.

While the variation in the characteristics of the titration curves for samples to which these methods are applicable is so great that general rules for sampling are almost impossible to formulate, the limits given in the methods (1, 2) are known to have given good results with the majority of the samples to which they have been applied.

The potentiometric method has generally been accepted without much comment in those cases where the

Table XII. Comparison of Free Acid Numbers Obtained for Dark Oils by Colorimetric and Potentiometric Methods

Oxidation Test No.	Time of Oxidation Hours	Acid No. Potentiometric method <sup>a</sup>	Acid No. Colorimetric method <sup>b</sup>	Oxidation Test No.	Time of Oxidation Hours	Acid No. Potentiometric method <sup>a</sup>	Acid No. Colorimetric method <sup>b</sup>
		Mg. KOH/g.					
1	0	1.19	Test fails <sup>c</sup>	5	8	1.4	0.6
	8	1.97	0.04		16	1.0	0.7
	16	2.31	0.80		24	2.3	0.7
	24	2.79	0.90		36	3.1	0.7
	36	3.00	1.00	6	8	0.6	0.2
2	8	9.5	6.6		16	1.4	0.4
	16	9.2	4.7		24	1.8	0.6
	24	7.1	4.1		36	2.2	0.9
	36	5.2	3.2	7	8	2.7	0.8
3	8	1.1	0.5		16	4.5	1.6
	16	1.2	0.5		24	4.9	1.5
	24	2.4	0.5		36	5.5	1.5
	36	2.2	0.5	8	8	2.7	0.8
4	8	6.9	4.3		16	4.1	0.6
	16	11.6	6.9		24	4.5	0.9
	24	15.4	7.2		36	5.1	2.0
	36	20.1	8.0				

<sup>a</sup> Designated A.S.T.M. D664-42T.

<sup>b</sup> Designated A.S.T.M. D663-42T.

<sup>c</sup> Test fails owing to inherent color of sample.



Table XIII. Comparison of Combined Acid (Saponification) Numbers Obtained for Dark Oils by Colorimetric and Potentiometric Methods

Sample <sup>a</sup>	Saponification Number or Combined Acidity			
	A.S.T.M. D94-39T colorimetric	A.S.T.M. D94-41T colorimetric	Total combined acidity, potentiometric	Strong combined acidity, potentiometric
		Mg. of KOH per gram		
1	3.5	4.5	3.4	0.2
2	30.6	30.7	30.9	0.7
3	1.2	1.7	1.6	0.6
4	3.6	4.6	4.5	0.3
5	40.2	30.7	28.5	0.5
6	1.0	1.6	0.9	0.3
7	3.4	4.2	4.2	0.2
8	28.8	30.1	28.5	0.3
9	2.5	4.8	3.6	0.5
10	4.9	7.8	7.8	0.6
11	25.2	27.1	27.6	21.3
12	31.4	33.1	35.2	24.7
Degras oil	117.3	121.8	132.0	10.1
Sperm oil	135.6	138.6	139.5	0.6
Sulfurized	167.7	161.6	163.4	26.4

<sup>a</sup> Samples 1 to 12 are oxidized oil samples.

titration curves show definite inflection points or breaks. The potential difference between the electrodes at the inflection point of a given sample varies from one electrode system to another and especially from one laboratory to another. While this variation has no appreciable influence when definite inflections are found, it is significant when the titration curves show no definite inflection points and the results are calculated from the volumes of base or acid required to produce a standard  $cG$  potential. Therefore, in the absence of definite breaks (less than 0.025-volt change per 0.10 ml. of 0.1  $N$  base) the titration is made to the potential found to be equivalent to the standard  $cG = 0.650$  volt, or  $cG = 0.236$  volt, by a test such as that suggested.

Even under good equilibrium conditions, titrations of some oxidized or used lubricating oils give curves that fail to reach the  $cG$  value expected for the given amount of base added. This lowering or flattening of the upper part of the titration curve is probably caused by the presence of certain materials having acidic characteristics that buffer the  $cG$  value in much the same way that certain phenols lower the pH of a dilute aqueous solution of a strong base. Except in the case of oils containing complex metallic salts or dopes, the lowering or flattening of the titration curve is probably not due to the consumption of the base by the acid complexes in a saponification or hydrolysis reaction. Furthermore, the abnormal lowering of the curve probably is not caused by reaction of the base with nonacid constituents of the sludge.

In general, inspection of the titration curves submitted by various laboratories using the potentiometric method indicates that, in many instances, too large increments of standard base were used and a sufficient length of time was not allowed for equilibrium between the increments. Reasonably satisfactory results have been achieved by using a short, constant equilibration period (0.5 to 1 minute) when small, constant increments (0.05 to 0.10 ml. of 0.1  $N$ ) of base are added, but erratic high results are obtained when large increments of variable size are used with short, constant equilibration periods. If in-

crements of variable size are used, as during a prolonged titration, more satisfactory results are obtained by allowing the electrode system to come to equilibrium after adding each increment. These considerations are especially important in the region of the end point. False breaks are sometimes manifested when a series of large increments is followed by several small increments and insufficient time is allowed for equilibrium.

The methods for free and combined acids and bases given in this paper have been applied to the analysis of over 5000 samples. They have been found extremely valuable in studies of the oxidation of petroleum products, and in the evaluation of a great number of materials. More recently, the methods have been adapted to a semimicro scale; equipment of a special nature has been designed for such small-scale work (21).

Isopropyl alcohol, c.p., was chosen as the solvent for the standard acid and base because it is one component of the titration solvent and because caustic solutions made from it are more stable than those made from ethyl alcohol. Special grades of isopropyl alcohol to which odorants may have been added are not recommended for the preparation of these reagents.

In the determination of acidity in complex mixtures, such as lubricating oil or organic distillates, any information which helps to identify the acidic constituents may prove valuable. The potentiometric titration gives such information, and, in addition, furnishes a record of just what took place during the analysis of any sample.

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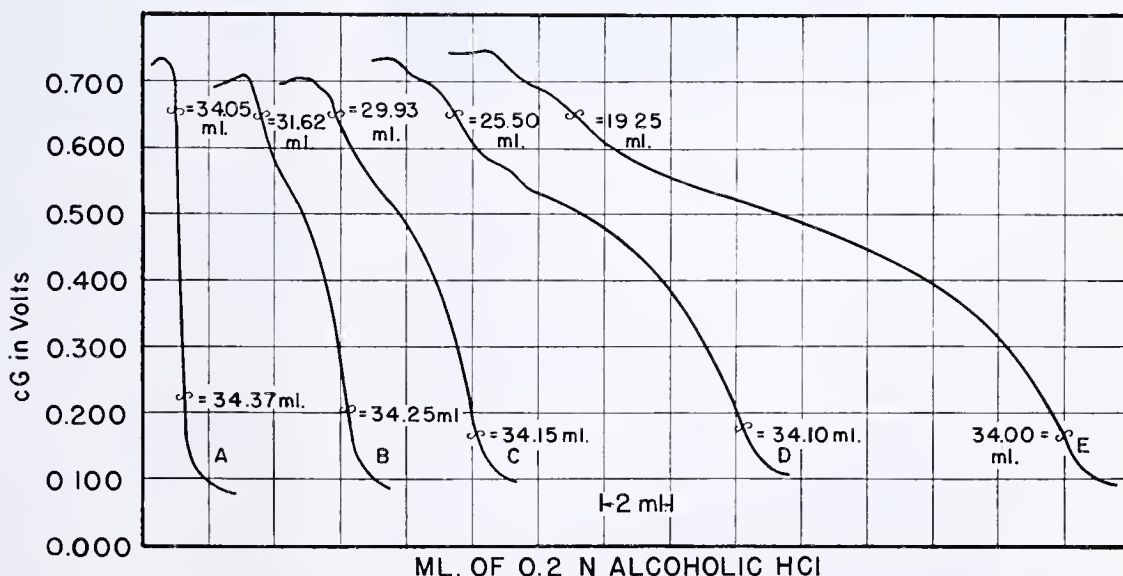


Figure 18. Influence of Sample Size upon Determination of Combined Acids and Bases

Potentiometric titration of basic materials remaining after saponification of following amounts of oxidized oil with potassium hydroxide: A 0.00, B 1.101, C 2.014, D 4.029, E 8.01 grams  
Titration cell. Glass electrode || calomel electrode  
Saponification medium. 40 ml. of benzene, 40 ml. of isopropyl alcohol containing approximately 7 milliequivalents of potassium hydroxide



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## Laboratory Evaluation of Corrosion-Inhibitive Pigments

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The importance of water condensation in failure of metal-protective paints by corrosion is discussed. Severe-service tests are differentiated from "accelerated" tests. Techniques are described for laboratory evaluation of the corrosion resistance imparted by metal-protective pigments. The preferred technique combines use of thin metal foil panels 0.0005 inch thick, single films or thin films offering minimum physical protection, and exposure under controlled water-condensation conditions.

THIS paper deals with methods for investigating the protective value of corrosion-inhibitive pigments in maintenance paints for metal surfaces, with emphasis on degree of inhibitive action rather than film durability.

Because of the many variables which may be encountered in service, the evaluation of pigmented coating compositions for the protection of structural steel surfaces against atmospheric corrosion represents a most difficult field of testing. For the same reason, apparently conflicting performance data are sometimes obtained in so-called practical tests.

Progress in the development of improved pigments and paints has accordingly been slow and the actual advances represented by new products have been uncertain. Honest differences of opinion may exist as to the relative merit of standard products which have been in extensive use over a number of years. Even after an anticorrosive pigment has been properly formulated into paint, according to existing laboratory tests, varied conditions in service may introduce factors that have a pronounced influence on the protective effectiveness of the system.

Important variables in the use of coating compositions include the metal painted, the condition of the surface (including rust and mill scale), film thickness, temperature and moisture conditions during painting and drying, contact with other paint films (old paint and new topcoats), and finally the several atmospheric conditions including moisture, corrosive chemicals such as salt, sulfur dioxide and others found in some atmospheres, heat, and sunlight. The investigator interested in development of metal-protective pigments faces an even more complex situation since, in addition, he must consider control pigments, combinations with other pigments, representative vehicles, and consistency as related to control of film thickness.

Evaluation techniques making possible a more intelligent selection of leads prior to the essential long-time fence and field tests are therefore of the utmost importance. Practical evaluations of the latter type extend over many years and are not well adapted for exploratory studies. In setting up preliminary laboratory tests, conditions should be selected on the basis of an analysis of the requirements to be met by a metal-protective paint film in accomplishing its objective—namely, prevention of

corrosion of the metal. Film requirements include (1) maintenance of continuity, (2) maintenance of adhesion to the metal, and (3) inhibition at the metal-film interface. The pigment influences each of these characteristics but is particularly important in relation to providing protection by passivation and/or exclusion of water and oxygen and/or control of film acidity.

In connection with the development of improved metal-protective pigments, techniques have been devised in this laboratory by which individual protective characteristics of a candidate pigment can be studied under controlled conditions to provide a basis for subsequent exposure work. These are not "accelerated tests", at least in the usual sense of the term, and in particular they are not accelerated tests in which some one factor has been exaggerated to a degree never met in practice in order to induce some kind of rapid failure. Thus, they are not to be considered in the category of salt-spray and continuous-immersion tests which are frequently used as accelerated tests and accepted as indicating performance under atmospheric conditions but which actually demonstrate performance under special conditions occasionally met in practice. Rather, they are to be considered a "severe-service" tests in which severe conditions which are most likely to cause failure in general service are brought into the laboratory and reproduced as faithfully as possible without intensifying any factor to a degree greater than that actually met in practice. For example, water continuously or frequently in contact with a paint film is an unquestioned factor in promoting corrosion failure. Frequent water condensation in practical service may cause failure of metal-protective paints which stand up satisfactorily under more favorable conditions. Rusting on the bottom

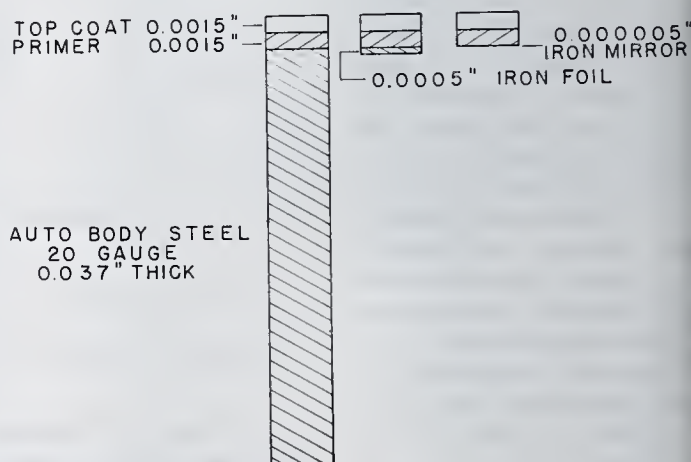


Figure 1. Relative Thickness of Panel Material Used in Testing Effectiveness of Metal-Protective Primers





**Figure 2. Miniature Iron Foil Panel Painted with Iron Oxide-Linseed Oil**  
 Left. Paint side after exposure. Right. Back of panel. Note underfilm corrosion

surface of steel beams, while sides and top are in good condition, a common occurrence which can be traced to condensation and hanging water droplets.

In setting up tests to establish corrosion-inhibitive effectiveness, the general rules have been (1) to use only conditions which are actually met in practice, (2) to apply the more severe conditions most likely to induce failure, and (3) to limit the evaluation so far as practicable to one cause of failure. In the techniques presented, prevention of underfilm corrosion has been emphasized. The ability of the pigment-binder combination to inhibit corrosion directly or to keep corrosion-inducing agents away from the metal is the property under study, with durability and adhesion either minimized or secondary. The importance of the latter two factors is clearly recognized, but they are secondary unless underfilm corrosion is controlled simultaneously.

With regard to the mechanism of corrosion, previous workers have firmly established several points which stand out from the maze of theories and isolated facts in the literature, and furnish guidance in test development. Atmospheric corrosion cannot proceed unless both water and oxygen are present. The process of corrosion may be accelerated by the presence of active acids or certain electrolytes. In service, the atmospheric oxygen factor may be considered constant and the moisture factor the primary atmospheric variable. Atmospheric acidity is undoubtedly involved in some severe failures in service—for example, acidity in the form of sulfur dioxide or trioxide (smoke) and carbon dioxide. Electrolytes may be very important in specific cases and must not be overlooked—for example, sodium chloride from sea water spray or other sources. Film acidity may also be involved. However, in the majority of cases, water appears to be the controlling factor in promoting corrosion under an intact film (excluding durability). Consequently, it was selected for emphasis in developing laboratory severe-service tests.

In studying the corrosion-inhibiting power of pigmented films, it is important to detect the first stages of corrosion and to follow its progress. To this end, a technique has been developed in which the normal metal panel is replaced by extremely thin metal foil representing the immediate surface of the ordinary panel and so thin that perforation occurs as soon as corrosion starts. This "thin foil technique" makes possible the study of inhibition under an intact paint film normally applied and without the introduction of abnormal influences.

In addition, two general techniques reflecting severe conditions occurring in practice have been selected with the previously stated general rules in mind. The first involves use of a relatively thin paint film, a condition which is often encountered in practice and which represents a severe test of protective power because of the greater permeability of the film to water. The second involves moisture condensation, the basis for which has already been discussed.

These three techniques will be described in greater detail, to-

gether with adaptations which introduce the further factors of weathering and prerusted steel surfaces.

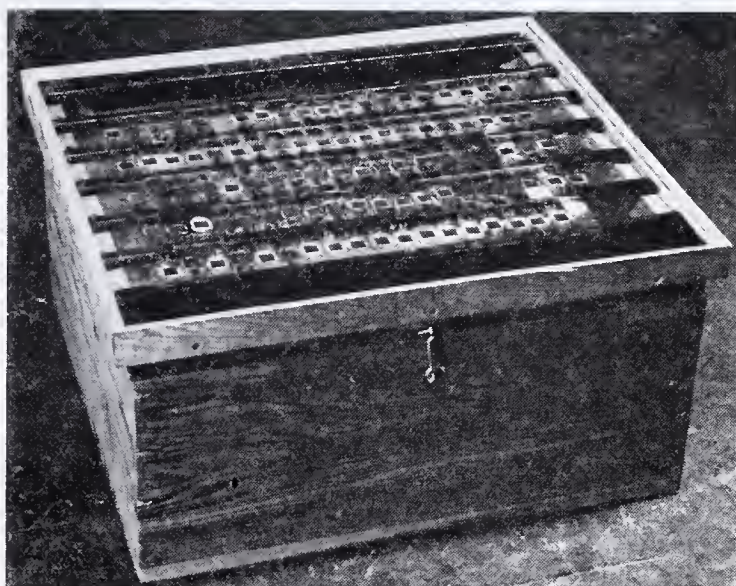
#### THIN FOIL TECHNIQUE

The general method comprises the painting of one side of a metal "panel" so thin that a minute amount of corrosion will be apparent from the back or uncoated side of the panel which is protected by sealing it with an inert resin on a transparent glass plate such as a microscope slide. Panels made up from iron foil about 0.0005 inch thick have been extensively used in development studies.

Figure 1 indicates the relative thickness of such thin panels as compared with ordinary 20-gage steel panels and with an ordinary paint film.

Corrosion usually becomes visible from the panel rear before it does through the paint film. This is particularly helpful with dark-colored primers and in cases where topcoats are applied over the primer film. In the case of blistering or questionable areas, it can be seen directly whether or not actual corrosion is involved. Figure 2 shows a painted miniature iron foil panel after an exposure; at left is the paint side and at right the extent of underfilm corrosion as seen from the back of the panel.

The thin foil technique can readily be extended to nonferrous metals such as aluminum and magnesium alloys.



**Figure 3. Indoor Water-Condensation Cabinet Open to Show Miniature Panels**

**PREPARATION OF THIN FOIL PANELS.** The thin iron foil used in this investigation was obtained from the American Platinum Works, Newark, N. J. The iron was described as "Electrolyt-Iron Heraeus-Vacuum-Schmelze A. Ca., Hanau, a.M., Germany", having the following elements in addition to iron: manganese, 0.030%; silicon, 0.030%; carbon, 0.010%; phosphorus 0.008%; sulfur, 0.016%.

The foil used has a thickness of the order of 0.0003 to 0.0005 inch. It is cut into miniature panels (0.625-inch squares) with a paper cutter after being placed within the fold of a sheet of paper. Before use, each miniature panel is examined for freedom from holes and blemishes. To protect from premature rusting, the small squares of foil are kept immersed in mineral spirits.

The technique used in mounting the miniature foil panels is as follows:

Clean microscope slides (3 × 1 inch) are laid on an electric hot plate and heated until the glass-to-metal adhesive, an inert ether resin of the type disclosed in U. S. Patent 2,060,715, melts and adheres when rubbed on the hot glass surface. Sufficient adhe-



sive is transferred in this manner to cover the central third of the glass slide with a thin film. The slide is then removed from the hot plate with a forceps and one of the 0.625-inch squares of foil is transferred to the central fluid resin area. Intimate contact of resin to metal is secured before the resin solidifies by pressing the metal foil with the end of a bar very slightly smaller in cross section than the piece of foil. If there is a tendency for the edge of the foil to turn up, a small spatula with turned up point is used to seal these edges by pressure. The mounted foil is ready for painting when cool. If painting is delayed, the mounted foil panel is kept in a desiccator to protect against atmospheric corrosion. The sealing resin must be inert to avoid the possibility of corrosion from this source, and it must retain its bond to glass in the presence of water. Turned up edges of the foil should be avoided, since they will later cut the paint film and end the test prematurely.

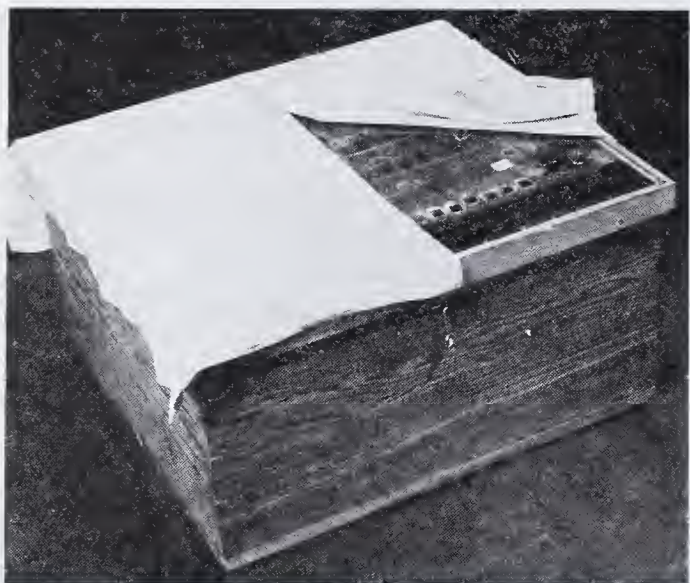


Figure 4. Indoor Water-Condensation Cabinet Covered by Glass Plate and Wet Towels

The miniature panel is painted with a flat 0.5-inch brush. Attention is given to securing a "normal" film thickness of about 0.0015 inch except where a test of the effectiveness of thin films is desired (see below). This is done by using a relatively "dry" brush and first painting out each system on a specimen area corresponding to the miniature foil panel. The amount of paint to be applied to the foil area is the amount necessary to give the required increase in weight on the test specimen area. When the effect of a topcoat is to be tested, the latter is applied over the primer in a similar manner after a suitable drying period. Almost insignificant amounts of paint materials are required, and it is therefore imperative that the brush used be clean of other paint and dry of solvents.

#### THIN FILM TECHNIQUE

Primed metal without topcoat is frequently exposed to atmospheric influences for long periods. Since this represents a severe test of inhibitive effectiveness, a single primer coat has been used in much of the routine testing of pigments in this laboratory.

Where a particularly severe, yet practical, test of inhibition is desired, the normal thickness of the primer film, 1.5 to 2 mils, is reduced by about 50%. This is legitimate, since practical painting practices frequently result in thin areas of film. Improper thinning of normally effective paints in service has been known to produce early failure for this reason. The film thickness factor is especially important if the primer is expected to be the sole protecting film over a long period of time, as is the case with shop-primed maintenance steel. For these reasons, it is frequently desirable to determine the performance of metal-protective pigments in a thinner paint film than would be recommended.

#### WATER-CONDENSATION TECHNIQUE

Because of the fundamental dependence of extent of corrosion on degree of moisture condensation, controlled water condensa-

tion in laboratory equipment has been used for evaluation of inhibitive power, selecting conditions which, although severe, represent commonly occurring conditions that cause corrosion in practice. Heavy condensation is a general phenomenon, existing wherever the temperature of the metal object drops below the dew point, and may be encountered wherever atmospheric humidity is relatively high, as in the vicinity of a stream or near the seacoast or in a damp basement or tunnel, or wherever warm days are followed by cool nights with rapid decrease in temperature. It occurs daily in the tropics and frequently in other regions. The degree of corrosion is also governed in large measure by the frequency of long damp periods. Choice of a wet/dry condensation cycle can be made in specific cases to adjust the severity of the exposure to correspond to that of any condition in any climate.

Two types of condensation tests have been used, an indoor test involving alternate periods of condensation and drying without weathering, and an outdoor test comprising alternate periods of condensation and exposure outdoors 45° south. Each contributes useful information and the two preferably should be run in parallel. Both sets of conditions are met in practice.

Two water-condensation cycles have been used in laboratory service tests. The "daily cycle" involves a wet period of water condensation at night and a dry period during the daytime, corresponding to the diurnal variation that often occurs. The "weekly cycle" of 4 days wet and 3 days dry is used to simulate the effect of frequently occurring damp periods. For the film-metal systems studied, corrosion was definitely more rapid for the weekly cycle than for the daily cycle, even though the wet fraction of the total cycle is about the same. Corrosion in the daily cycle is much more severe than in the much shorter cycles of some "accelerated tests" where the wet to dry change takes place considerably more frequently (such as at 15- to 60-minute intervals), there being less chance for either a dry or wet equilibrium condition to be developed in the film. The exact significance of the dry portion of the cycle has not been established. However, it affords an opportunity for migration of water-soluble components of the film which theoretically may occur.

The results of indoor water condensation tests should be interpreted only in the light of the purpose for which the test was designed, to show the behavior of film systems when used under conditions involving relatively prolonged water condensation and no exposure to outdoor weathering. The indoor tests do not show the specific effects of sunlight on the ultimate durability of the system but they do emphasize the inhibitive value of the film.

Outdoor exposure at 45° south can be included intermittently



Figure 5. Outdoor Water-Condensation Cabinet





Figure 6. Typical Underfilm Corrosion

Rear view of painted miniature iron foil panels after exposure

Corrosion of simple single-pigment-linseed-oil primer systems after exposure in indoor water-condensation cabinet under selected conditions

with the cycle for the indoor box, where this type of information is desired.

**WATER-CONDENSATION CABINET, INDOORS.** The panels are exposed painted side downward between bars suspended over the top of wooden boxes about 24 × 24 × 12 inches deep (see Figures 3 and 4). The top edge of the box is recessed to receive a snugly fitting plate-glass cover, which is supported about 0.125 inch over the panels. The box is waterproofed by waxing inside. A wide tray containing water covers the bottom of the box and may be reached through a door in the side. If the number of exposure panels is insufficient to cover the top area of the box completely, "dummy" panels are used to fill up the space, so that the system is a closed one even before the glass plate is put in place. A wet towel is placed over the glass plate and kept wet. Continuous evaporation from the towel maintains the temperature of the panels in the box at or below the dew point. In this closed water-saturated system, fine water droplets condense and remain on the painted side of the panels. The box is deep to facilitate regulation of the H<sub>2</sub>O of the condensed water if it is desired to investigate the effect of acid dews (such as with sulfur dioxide).

A somewhat simpler although less flexible arrangement is one in which the deep box is replaced by a relatively shallow tray, and the panels are directly sealed (paint side exposed) to the bottom of the large glass cover with a beeswax-rosin mixture. The cover may be kept cooled by a thin layer of water retained thereon by a shallow wax dam around its edges or by the above towel arrangement.

**WATER-CONDENSATION CABINET, OUTDOORS.** The general arrangement for outdoor exposure is essentially the same as that of the indoor box, with the added feature that the cover is hinged and the panels are fastened thereto in a manner permitting them to be exposed at 45° facing south by swinging the cover back on its hinge 135° from the normal face-down position (see Figure 5). The top of the box is plate glass sealed in the wood frame so as to provide a tray. In the summer months, condensation is promoted on the panels by the cooling induced by evaporation from a 0.25-inch layer of water maintained in this tray when the box is closed. During other seasons when the temperature is low outdoors, no cooling water is needed but several low-wattage immer-

sion heating coils are required in the interior water tray to ensure the necessary temperature differential for condensation. The water is maintained about 10° F. above the air temperature when it is necessary to use the heaters.

**EXPOSURE CYCLE.** For the indoor condensation tests, two wet/dry cycles have been used. The daily cycle corresponding to that of the outdoor box is not so effective in producing corrosion as is the weekly cycle consisting of 4 days of continuous condensation and 3 days dry. This latter period has been used to the greatest extent, inasmuch as it is considered to simulate the effect of frequently recurring condensation during humid periods or in damp locations. The daily cycle corresponds more nearly to heavy condensation conditions in the absence of direct sunlight.

The outdoor arrangement is best adapted to a daily cycle with water condensation at night and exposure at 45° south during the day. For convenience, a wet/dry cycle of 15/9 hours has been used in the work to date. A weekly cycle comparable to that used in indoor tests appears to have promise but has not been explored thoroughly.

## PERFORMANCE

**REPRODUCIBILITY OF RESULTS.** As in all corrosion evaluation, it is advisable to make at least duplicate and preferably triplicate panels. General experience has been that results with the miniature panels are readily reproducible. Performance has been much more consistent than for ordinary large panels on outdoor rack tests.

**TIME REQUIRED FOR TESTS.** The time required for significant results depends on the efficacy of the protective systems and on

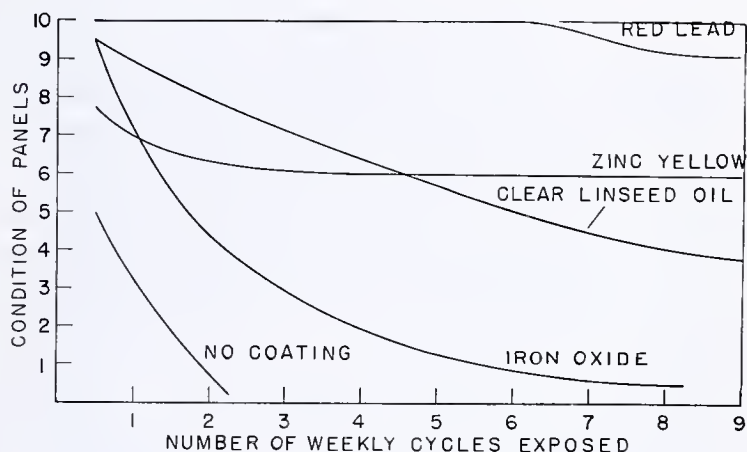


Figure 7. Primer Only, 25° C.

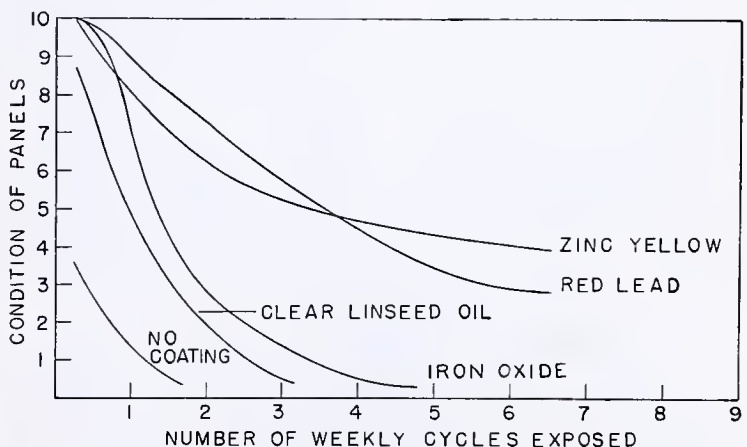


Figure 8. Primer Only, 25° C., SO<sub>2</sub> Condensate (pH 5.0)



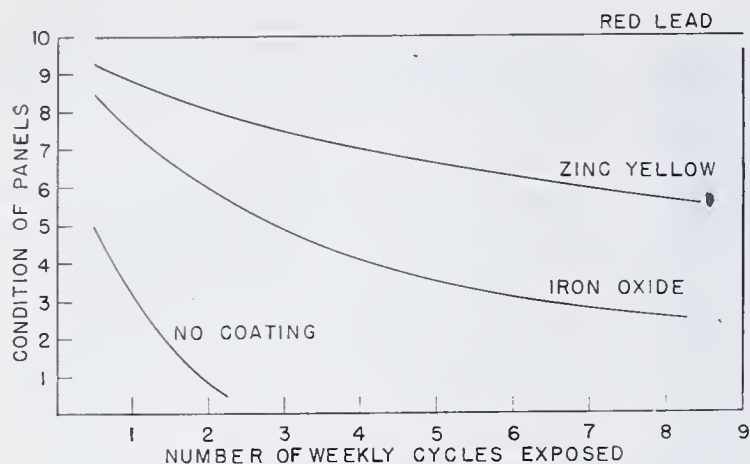


Figure 9. Primer and Topcoat, 25° C.

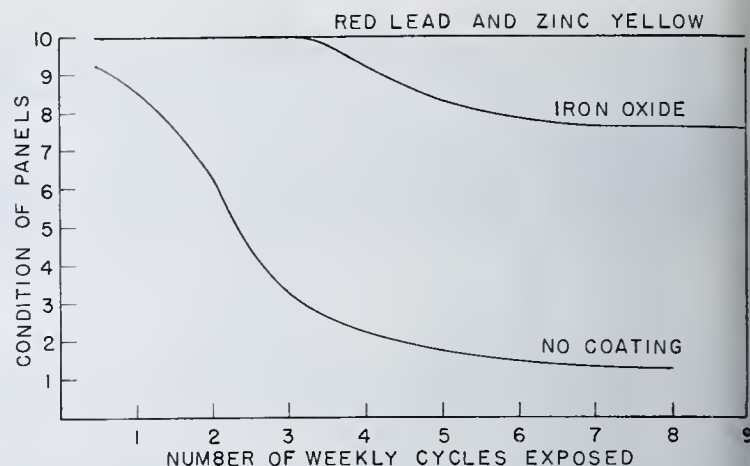


Figure 10. Primer and Topcoat, 5° C.

the cycle chosen. In the case of an outdoor water-condensation box with a daily cycle, differences among the poorer systems are often apparent within a month or two, while some systems show failure only after 10 months or so. In the case of the more severe condensation test using the weekly cycle, similar failure occurs indoors within shorter time intervals—i.e., within the corresponding number of weeks.

**METHOD OF GRADING.** The panels are graded on a numerical basis after visual examination of both the front and the back of the panel. Inasmuch as the early incidence of corrosion is of interest, a grading code has been used that emphasizes slight initial changes. A grading of 10 represents no rusting, whereas 0 represents complete failure. A grading of 9 denotes very slight (even questionable) corrosion, while 8 indicates slight but definite corrosion, and 7 corresponds to failure of about 10% of the metal surface area. Gradings from 7 to 0 are in proportion to the surface area remaining unattacked—i.e., from 90 to 0%.

#### TYPICAL RESULTS

The paints used to illustrate results are simple experimental compositions consisting of single pigments in refined linseed oil at equal pigment volumes, in which no attempt has been made to develop the maximum protective effectiveness possible by correct formulation. Figures 7 to 11 present corrosion data obtained on them by one of the above-described techniques, while Figure 6 shows typical underfilm corrosion on a number of miniature panels. All results shown are for thin foil panels exposed in the indoor condensation cabinet using the weekly cycle of moisture condensation. The effects of temperature, topcoat, and use of a slightly acid (sulfur dioxide) condensation are shown. Three commercial grades of pigments, red lead, zinc yellow, and iron oxide, are used to illustrate the course of corrosion under these different conditions. Red lead and zinc yellow were selected as typical inhibitive pigments. Iron oxide was selected inasmuch as it has not ordinarily been considered as an inhibitive pigment, its extensive use being due to other factors. Tests have indicated wide variation in the behavior of different samples of iron oxide.

**THICK PANELS—PRERUSTED PANELS.** In certain instances, it will be possible to use panels of ordinary thickness—for example, when studying the corrosion of very thin primer films without topcoat or iron surfaces where corrosion often is visually apparent on the paint side of the panel after a relatively short exposure to moisture condensation. Use of thicker panel material is obligatory when using prerusted steel surfaces.

When panel material of ordinary thickness can be employed, it is convenient to use simple 3 × 1 inch panels. Full-scale panels, say 12 × 4 inches, may be used if desired.

**IRON MIRRORS.** The idea of thin panel material has also been carried to an extreme by the use of translucent iron mirrors.

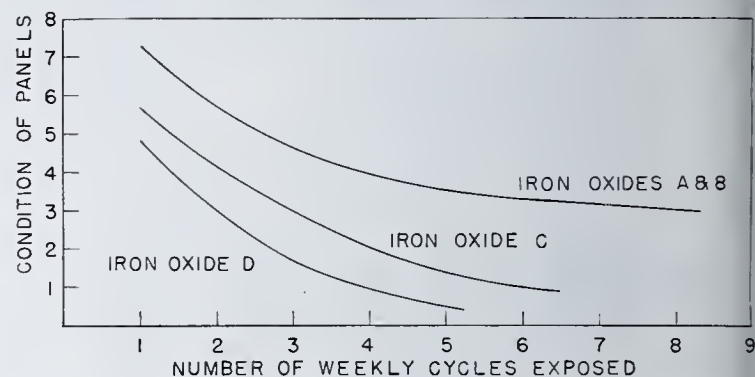


Figure 11. Primers, Different Grades of Iron Oxide, 25° C.

This system is so sensitive that it has not to date been useful from the standpoint of the objective of this paper—namely, evaluation of practical metal-protective pigments. However, the technique itself is briefly described because of its possible utility as a tool in studying theoretical aspects of the effect of pigments in preventing corrosion.

Translucent iron mirrors were prepared by evaporating relatively pure iron on a heated tungsten filament under high vacuum, the mirror being formed by condensation of the iron vapor on a 3 × 1 inch microslide in the evacuated system. Mirrors having a thickness of the order of 0.000004 inch (1000 Å.) were selected, selection being on the basis of visual density of the mirror film. The thickness has been checked by an interferometric method and by a colorimetric analysis for iron.

The iron mirror film is relatively stable to corrosion in the ordinary laboratory atmosphere. Under high humidity conditions, however, the film fails. Extremely thin mirrors are unsuitable because they fail by microcracking and microcurling at high humidity. Thicker, but still translucent, mirrors show the typical micro "pitting" type of corrosion when exposed for a week or two in an atmosphere of high humidity. An area of bright yellowish-brown rust appears at or near the center of the pit and appears to grow at the expense of the retreating iron mirror.

#### ACKNOWLEDGMENT

Acknowledgment is made to the Pigments Department of E. I. du Pont de Nemours and Company, Inc., which supported this work and provided for the photographic reproduction of the outdoor water-condensation cabinet at the Newark, N. J., laboratory. Appreciation is expressed to members of staffs of the Pigments Department and of the Chemical Department for assistance in experimental work.

PRESENTED before the Division of Paint, Varnish, and Plastics Chemistry at the 106th Meeting of the AMERICAN CHEMICAL SOCIETY, Pittsburgh, Pa. Contribution 196, Chemical Department, E. I. du Pont de Nemours and Company, Inc.



# Analysis of Soap-Synthetic Detergent Mixtures in Bar Form

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A procedure for the analysis of commercial soap-synthetic detergent mixtures is proposed which has given sufficiently accurate and reproducible results. The sample is extracted with 95% ethyl alcohol to remove the major portion of the active ingredients, followed by solution of the alcohol-insoluble salts in water and precipitation of them by the addition of excess ethyl alcohol. Soap, fatty matter, and alcohol-soluble chlorides are determined directly, synthetic detergent being determined as the difference between total alcohol-soluble matter and the sum of soap, fatty matter, and alcohol-soluble chlorides.

**A** BAR form of soap-synthetic detergent mixture has recently come into wide use by the Navy for general cleaning in sea water. Several publications have described the use of soap-synthetic detergent mixtures as replacements for coconut oil soaps which are no longer available in large quantities (3, 11, 12). Methods for analyzing these mixtures are being used by manufacturers for their own products, but as yet, except for a method contained in a government specification for bar form of salt-water detergent (2), no general procedure for the analysis of such mixtures has appeared in the literature. Inasmuch as the Navy Department is a large consumer of soap-synthetic detergent mixtures in bar form which must conform to specification requirements, it is important that an accurate method of analysis be available. This paper presents a sufficiently accurate and reproducible procedure for the analysis of soap-synthetic detergent mixtures in bar form, which was developed after an investigation of the literature and various procedures submitted by several soap and synthetic detergent manufacturers.

Methods for the direct determination of synthetic detergents have been reported (1, 4-7, 10), but none has been applied to the analysis of soap-synthetic detergent mixtures. Moreover, these methods are applicable only to those detergents whose exact structure is known, and cannot be used for commercial soap-synthetic detergent mixtures in which the synthetic portion is not identified and may consist of a mixture of indefinite composition.

Soap-synthetic detergent mixtures of the type useful for salt-water cleaning consist of an active portion (soap and synthetic detergent) and an inactive portion (inorganic salts, moisture, and fatty matter). The term "fatty matter", as used in this paper, refers to all constituents preferentially soluble in petroleum ether; this will normally include hydrocarbons, fats, and free fatty acids. The term "active ingredient", as used in this paper, is applied to the alcohol-soluble portion corrected for determined impurities. The present method is based on the separation of active ingredients from inorganic salts by means of ethyl alcohol, and the subsequent determination of soap, fatty matter, and sodium chloride in the alcohol-soluble portion. Synthetic detergent is calculated as the difference between total alcohol-soluble matter and the sum of soap, fatty matter, and alcohol-soluble sodium chloride.

## PROCEDURE

**ANHYDROUS, SALT-FREE SODA SOAP.** Weigh to the nearest milligram a 2-gram sample of the soap-synthetic detergent bar in a tared 300-ml. Erlenmeyer flask. Add 50 ml. of water and 50 ml. of 95% ethyl alcohol. (Formula 2B or 30 alcohol may be used if 95% ethyl alcohol is not available.) Warm on the steam bath until no further solution takes place. Cool, add 5 drops of methyl orange indicator, and titrate with 0.5 *N* sulfuric acid to a pink color. Add 5 to 6 ml. in excess. Transfer the contents of the flask to a 500-ml. separatory funnel, washing out the flask with 50 ml. of water, then with 50 ml. of ethyl alcohol. Add the washings to the separatory funnel. Extract the fatty acids and fatty matter 4 times with petroleum ether, using 40-ml. portions.

Combine the petroleum ether extracts and wash with small portions of distilled water until the water washings are no longer acid to methyl orange.

Transfer the petroleum ether extracts to the original 300-ml. tared flask, filtering if necessary, and wash the separatory funnel with 2 small portions of petroleum ether, adding the washings to the flask. Evaporate the petroleum ether on the steam bath, then dry in an oven at 100° to 105° C. to constant weight. Cool and weigh as fatty acids plus fatty matter.

Dissolve the fatty acids and fatty matter in 50 ml. of neutral ethyl alcohol with warming; add phenolphthalein indicator and titrate with standard alcoholic 0.1 *N* sodium hydroxide.

Calculate the per cent of soap plus fatty matter as follows:

$$\begin{aligned} &\text{Per cent of soap +} \\ &\quad (\text{ml. of NaOH} \times \text{normality factor} \times 0.022) + \\ \text{fatty matter} &= \frac{(\text{weight of fatty acids + fatty matter})}{\text{weight of sample}} \times 100 \end{aligned}$$

Calculate per cent of soap by subtracting from this value the per cent of fatty matter determined later.

**ALCOHOL-INSOLUBLE MATTER.** Weigh to the nearest milligram a 2-gram sample in a 250-ml. beaker, add 100 ml. of 95% ethyl alcohol, cover the beaker, and heat on the steam bath with frequent stirring and maceration of the sample until it is completely disintegrated. Let settle and filter through a tared Gooch crucible with suction into a tared 300-ml. Erlenmeyer flask, retaining as much of the residue as possible in the beaker. Repeat this extraction 3 times with 25-ml. portions of hot 95% ethyl alcohol, each time retaining as much of the residue as possible in the beaker. Finally, evaporate any remaining alcohol and dissolve the residue in the smallest possible quantity of hot distilled water (5 ml. should be sufficient). Reprecipitate the alcohol-insoluble matter by slowly adding with vigorous stirring 50 ml. of 95% ethyl alcohol. Heat the solution to boiling on the steam bath, filter, and transfer the precipitate to the Gooch crucible, washing several times with 95% ethyl alcohol. Dry the crucible at 100° to 105° C. to constant weight and calculate the per cent of alcohol-insoluble matter. Reserve the combined filtrate and washings.

**ALCOHOL-SOLUBLE MATTER.** Evaporate the filtrate and washings obtained in the determination of alcohol-insoluble matter to dryness on the steam bath. Heat the residue to constant weight at 100° to 105° C. and calculate the per cent of alcohol-soluble matter.

**FATTY MATTER.** Dissolve the alcohol-soluble matter in a mixture of 50 ml. of water and 50 ml. of 95% ethyl alcohol, warming if necessary. Transfer the solution to a 500-ml. separatory funnel, washing out the flask with 50 ml. of water, then with 50 ml. of 95% ethyl alcohol. Cool and extract fatty matter 4 times with petroleum ether, using 25-ml. portions. Combine the petroleum ether extracts and wash 4 times with 10-ml. portions of 0.2 *N* sodium hydroxide, adding the washings to the alcoholic solution which is reserved for the determination of chlorides. Finally, wash the petroleum ether extract with small portions of water until the water washings are no longer alkaline to phenolphthalein. Transfer the washed petroleum ether extract to a tared 300-ml. Erlenmeyer flask, washing out the separatory funnel with 2 small portions of petroleum ether, and add the washings to the flask. Evaporate the petroleum ether on the steam bath and dry the residue at 100° to 105° C. to constant weight. Calculate the per cent of fatty matter.

**CHLORIDES IN ALCOHOL-SOLUBLE MATTER.** Add 15 ml. of 20% magnesium nitrate solution to the alcoholic solution remaining after the determination of fatty matter. Heat on the steam bath until the precipitate is coagulated, filter, and wash thoroughly with water. Make the filtrate acid with dilute nitric acid and determine chlorides by the Volhard method.

**SYNTHETIC DETERGENT, BY DIFFERENCE.** Calculate the per cent of anhydrous, salt-free, synthetic detergent as follows:

$$\% \text{ synthetic detergent} = (\% \text{ alcohol-soluble matter}) - (\% \text{ soda soap} + \% \text{ fatty matter} + \% \text{ NaCl in alcohol-soluble matter})$$

## DISCUSSION

The usual method for analyzing commercial synthetic detergents consists in the separation of alcohol-soluble and insoluble portions by extraction with ethyl alcohol. The alcohol-soluble portion is then generally considered to be "active synthetic deter-



Table I. Adsorption of Synthetic Detergents

(Values represent average of 4 individual determinations, expressed as per cent of total sample)

Sample	Loss in Weight on Ignition of Alcohol-Insoluble Residue		Alcohol-Soluble Matter Recovered from Precipitate of Alcohol-Insoluble Salts		Synthetic Detergent	
	Method I <sup>a</sup>	Method II <sup>b</sup>	Method I	Method II	Method I	Method II
1	4.1	0.9 <sup>c</sup>	8.3	0.0	37.1	46.3
2	2.2	0.1	5.0	0.0	34.9	40.9
3	1.5	0.2	4.0	0.0	30.5	34.7
4	4.9	0.3	5.7	0.0	47.2	53.8
5	1.5	0.1	1.4	0.0	19.9	22.0
6	2.8	0.4	3.4	0.0	25.6	29.7
7	1.8	0.3	3.6	0.0	22.8	26.9
8	1.2	0.2	3.2	0.0	17.1	21.1

<sup>a</sup> Method I, no reprecipitation of alcohol-insoluble matter.<sup>b</sup> Method II, reprecipitation of alcohol-insoluble matter.<sup>c</sup> High result probably represents loss of water of hydration of borax which is present in this sample.

of eight samples by the nonprecipitation and precipitation methods are also given in Table I.

Samples 1, 2, 3, and 4 were four commercially available synthetic detergents. Sample 1 was the sodium salt of a sulfonated straight-chain hydrocarbon; samples 2 and 4 were sodium salts of alkyl aryl sulfonates; sample 3 was the sodium salt of a sulfonated glyceryl ester of a straight-chain fatty acid. Samples 5, 6, 7, and 8 were mixtures in bar form of soap and the above synthetic detergents.

In addition to giving incomplete separation of alcohol-soluble matter from alcohol-insoluble salts, most of the methods investigated gave incorrect values in the determination of soap, chlorides, and fatty matter. As the synthetic detergent is determined by difference, the values reported for it may be correspondingly inaccurate. Experience with the submitted meth-

ods showed that soap values tended to be low and fatty matter values high, while the difficulty in determining the end point in the chloride determination led to either high or low values.

It is believed that the low values obtained for soap are due to the incomplete decomposition of the soap and consequent incomplete extraction of the fatty acids when mineral acid is added only to a methyl orange end point. Judging from the results obtained, the small excess of acid added in the proposed method is

sufficient to decompose the soap completely, but insufficient to decompose the synthetic detergents used.

The high values obtained for fatty matter are believed to be due to solution of acid soaps in the ether used for extraction. Such acid soaps cannot be removed by washing with water alone (8). Washing the petroleum ether solution of the fatty matter with dilute alkali seems to overcome this difficulty.

#### APPLICATION OF METHOD TO KNOWN MIXTURES

Inasmuch as no referee method exists for the analysis of commercial soap-synthetic detergent mixtures in bar form, the present method was tested by analyzing mixtures of known composition, containing soap, synthetic detergent, sodium sulfate, fatty matter, and sodium chloride.

The mixtures were prepared by dissolving the constituents in 50% alcohol, diluting to a definite volume, and taking aliquots for the analyses.

The fatty matter used in the mixtures was obtained by extraction of the synthetic detergent in the commercially available form with petroleum ether in a Soxhlet extractor. The filtered petroleum ether solution was then washed thoroughly with 50% alcohol, evaporated, and dried to constant weight at 105° C. and this extract was used to furnish the fatty matter.

The material left in the Soxhlet after extraction with petroleum ether was extracted with absolute alcohol to remove alcohol-soluble synthetic detergent. The alcoholic solution was then evaporated and dried to constant weight, and after being corrected for sodium chloride present was used in the preparation of known mixtures.

The soap used in the mixtures was obtained by first extracting solutions of two commercial soaps with petroleum ether to remove unsaponified and unsaponifiable matter. The residue obtained on drying was dissolved so far as possible in alcohol and the filtered alcoholic solution evaporated to dryness and to constant weight. This residue, corrected for glycerol and sodium chloride present, was used in the mixtures.

Table II gives values obtained by the proposed method on 5 known mixtures. Synthetic detergent A is the sodium salt of a sulfonated glyceryl ester of a straight-chain fatty acid; B and C

Table II. Analyses of Known Soap-Synthetic Detergent Mixtures

Determination	Mixture 1		Mixture 2		Mixture 3		Mixture 4		Mixture 5	
	Present %	Found %	Present %	Found %	Present %	Found %	Present %	Found %	Present %	Found %
Alcohol-soluble matter	77.7	77.8	67.8	67.9	74.0	74.0	81.1	81.1	74.8	74.5
Alcohol-insoluble matter	22.3	22.4	32.2	32.0	26.0	26.0	18.9	18.8	25.2	25.2
Soap + fatty matter	44.5	44.3	43.2	43.1	41.3	41.2	48.6	48.9	46.2	46.2
Fatty matter	1.3	1.1	4.4	4.4	2.0	2.0	1.2	1.2	0.8	0.8
Soap	43.2	43.2	38.8	38.7	39.3	39.2	47.4	47.7	45.4	45.4
Chlorides	1.2	1.3	4.7	4.7	2.6	2.6	1.1	1.1	0.8	0.8
Synthetic detergent	32.0	32.2	19.9	20.1	30.1	30.2	31.4	31.1	27.8	27.5
Sum of alcohol-insoluble matter, chlorides, soap, synthetic detergent, and fatty matter	100.0	100.2	100.0	99.9	100.0	100.0	100.0	99.9	100.0	99.7
Type of soap used	A		Toilet Soap B		C		D		Oleic acid soap E	
Synthetic detergent used										

gent". Low results were always obtained by this procedure in this laboratory. In the analysis of soap-synthetic detergent mixtures by the above alcohol-extraction method, low results for synthetic detergent were also obtained. These low results are caused by the fact that some synthetic detergent is adsorbed by the alcohol-insoluble salts: (1) When the residue of alcohol-insoluble matter was dried in an oven at 105° C. and then ignited at 500° C., the loss in weight in some instances amounted to almost 5% of the total sample. (2) The residue of alcohol-insoluble matter foamed in water, indicating the presence of some active material, which might be soap or synthetic detergent. (3) The residue of alcohol-insoluble matter formed a very turbid solution upon the addition of Pedersen's reagent, which does not react with soaps or the salts present (9).

Investigation showed that the adsorption of synthetic detergent by the alcohol-insoluble residue can be very greatly reduced and the separation made more complete by a procedure which involves initial extraction of the sample with 95% ethyl alcohol to remove the major portion of the alcohol-soluble matter, followed by solution of the residue in the smallest possible quantity of water and precipitation of the inorganic salts by the addition of ethyl alcohol.

To determine directly the amount of adsorption, samples were extracted with ethyl alcohol, and the residue of alcohol-insoluble matter was filtered on a Gooch crucible, dissolved in the minimum amount of hot water, and reprecipitated by the addition of excess ethyl alcohol. The precipitate was filtered off and the filtrate evaporated to dryness and weighed. This weight represented the amount of synthetic detergent which could be recovered from the thoroughly extracted residue by solution and reprecipitation.

The results in Table I show that the adsorption of synthetic detergent by the alcohol-insoluble residue, as indicated by (1) loss in weight upon ignition of the alcohol-insoluble residue and (2) recovery of synthetic detergent from the alcohol-insoluble residue, can be markedly reduced when the reprecipitation procedure is employed. Values for the synthetic detergent portion



Table III. Analysis of Known Soap-Synthetic Detergent Mixture Prepared in Bar Form

Type of soap, toilet soap. Synthetic detergent, sodium salt of a sulfonated amide of a straight-chain fatty acid)

Determination	Present %	Found %
Alcohol-soluble matter	72.1	72.0
Alcohol-insoluble matter	27.9	27.7
Synthetic detergent	23.4	23.3
Soap	46.6	46.5
Chlorides	2.1	2.2
Fatty matter	0.0	0.0

are sodium salts of alkyl aryl sulfonates; D is the sodium salt of a sulfonated amide of a straight-chain fatty acid; E is the sodium salt of a sulfonated straight-chain hydrocarbon.

In order to guard against the possibility that results obtained by the proposed method on a mixture in bar form, as opposed to mixtures reported in Table II, might be incorrect, an additional sample of known composition made up in bar form was analyzed. The results as given in Table III are of satisfactory accuracy. The figures given are on the anhydrous basis.

#### ACKNOWLEDGMENT

The authors are indebted to R. C. Hughes and C. W. Schroeder, present address, Continental Foods Corp., Hoboken, N. J.) of this laboratory for their many helpful suggestions, and also to the following for suggested methods: Jay C. Harris, Monsanto

Chemical Company; Armour and Company; American Cyanamid and Chemical Corporation; General Dyestuff Corporation; L. F. Hoyt, National Aniline Division, Allied Chemical and Dye Corporation; and Colgate-Palmolive-Peet Company. They are indebted to Lever Brothers Company for suggesting the use of 50% alcohol solution for dissolving the sample, and to J. H. Shipp and Harold Jones of E. I. du Pont de Nemours and Company for a suggested method of analysis and for supplying the sample of Pedersen's reagent.

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THE views in this article are those of the authors and should not be construed as the official views of the Navy Department.

## A Carr-Price Reagent Dispenser

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THE saturated chloroform solution of antimony trichloride, used in the chemical determination of vitamin A, must be kept under anhydrous conditions at all times, and provision must be made for rapid dispensing of accurately measured volumes.

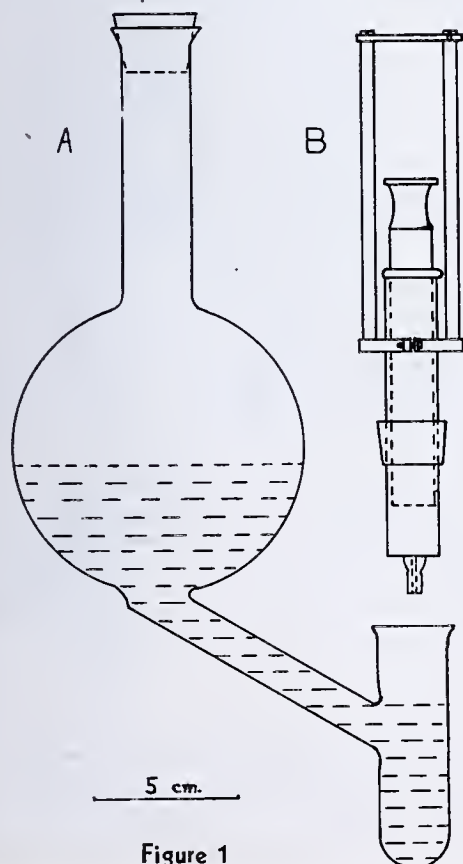


Figure 1

An ordinary pipet is unpleasant to use with this solution and is slow in delivery. The apparatus described serves to solve the problem where a considerable number of determinations are to be made.

A constant-level reservoir for the reagent (Figure 1, A) consists of a 300-ml. flask (or other convenient size) with an angular bottom offset leading to a side-arm reservoir wide enough for insertion of a 10-ml. Luer hypodermic syringe. To fill the reservoir the small one is stoppered and the solution is added to the large one, which is then securely stoppered. The liquid level in the small reservoir remains constant when liquid is withdrawn therefrom.

The solution is removed in measured volume by a hypodermic syringe (Figure 1, B) with needle removed, fitted with a brass collar which can be clamped on the syringe barrel. A wrapping of surgical tape on the barrel forms a firm cushion for the collar. Two vertical rods are soldered to the collar and the limiting distance to which the syringe plunger may be withdrawn is governed by a swiveling top bar which may be turned aside when the plunger is to be removed. A stopper around the barrel assures its suitable depth of immersion in the small reservoir and excludes contact between solution and atmospheric moisture. A pipet of similar principle but not suited to the present purpose was described by Krogh (1). A paper which appeared after submission of this note includes another design of apparatus for the same purpose (2).

For accurate measurement the plunger must be inserted in definite position of orientation with respect to the barrel. This is maintained by suitable markings on the syringe parts.

Tests, using water, gave four successive delivery weights of 9.02, 9.05, 9.03, and 9.04 grams. No tendency for the syringe orifice to drip during transfer was encountered. The syringe should not be left overnight without washing, as there is danger of freezing due to deposition of antimony trichloride. Rubber stoppers should be changed periodically because of attack by the reagent. The solution should be kept in the reservoir only during its actual use to avoid possible contamination from the rubber stoppers.

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# Determination of Carbon by the Low-Pressure Combustion Method

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The apparatus for the low-pressure combustion method of determining carbon in iron and steel has been redesigned to increase the speed of manipulation. Several thousand determinations showed results in good agreement with Wooten and Guldner's form of the apparatus. A detailed description of the equipment and its manipulation is given, together with an account of experimental studies on the method. Results are also shown for the carbon content of copper.

THROUGH the kindness of Wooten and his associates, the method of carbon analysis described in his recent paper (13) was shown to the authors in the spring of 1938. The desirability of securing accurate data on the carbon content of silicon steels induced them to investigate the possibilities of this method for types of steel which consist largely of iron with silicon ranging from 3 to 6%. Since that time about five thousand determinations of carbon have been run in this laboratory by that method, and this paper reports experience with it. The general problem of determining the carbon in iron and steel has been well discussed by Lundell, Hoffman, and Bright (?). In most general use for irons and steels is the combustion method (pp. 157 ff.), in which the steel is burned in a stream of oxygen and the resulting carbon dioxide is absorbed in a suitable train. There are many sources of error in this method, some of which are not easily controlled. These are reflected in the relatively large blank, which is difficult to reduce when dealing with small amounts of carbon and often equals or exceeds the amount to be determined. Measurement of the carbon dioxide formed by combustion of the steel has been one of the chief sources of error. It is upon this point in the procedure that many efforts (6) have been directed to reduce the error involved. Further comparisons of these methods are given below.

## APPARATUS

The earliest form of the authors' apparatus is similar to Wooten and Guldner's and is shown in Figure 1 with  $Z$  joined to  $Z'$  and the intermediate traps omitted, and without the cutoff on the combustion vessel. Various modifications have been made of the equipment, which at the present time is used in the form shown in Figure 2. This form requires the use of only two mercury wells, thus reducing the amount of mercury required by a factor of 2. As mercury is a critical costly material at the present time, it is expected that the McLeod gage can be replaced by a Pirani gage for measuring the concentration of carbon dioxide produced, with a consequent saving in mercury and the convenience of a direct-reading instrument.

Although Figure 2 shows only a single unit, the authors actually construct four units to operate from a common oxygen supply system (Figure 3). A single auxiliary vacuum serves for all four, and whereas each unit requires one mercury diffusion pump, the units are joined to operate with a common fore pump. Four units are all a single operator can conveniently manage. Just outside the picture at the left, symmetrically arranged with respect to the oscillator, is another unit of four. Both receive their power from the 5-kilowatt oscillator shown behind the operator.

In describing the apparatus, manipulation, and results, only the differences from the procedures described by Wooten and Guldner are emphasized; their publication (13) should be consulted for the elaboration of details. To facilitate comparison between the two papers, the same symbols are used in Figures 1 and 2 to designate parts and equipment functionally the same.

**PURIFICATION OF OXYGEN.** The catalyst is contained in a Pyrex tube and it has not been necessary to use the quartz tube shown in Wooten and Guldner's apparatus. This catalyst is active enough at 400° C. to oxidize all the carbonaceous materials occurring in the authors' oxygen gas. The palladium black catalyst was prepared by the method described by Farkas and Melville (3, p. 338) by precipitation on broken pieces of Alundum and

was loaded into the tube after preparation. Operation of the catalyst at this low temperature considerably lengthens the life of the furnace used to heat the tube. The amount of oxygen consumed is small; hence, the gas from an ordinary pressure cylinder will last a long time and is more constant in composition than line oxygen.

Although the authors use liquid nitrogen for cooling their traps, and liquefied the oxygen in their first experiments, they found that they could obtain oxygen of sufficient purity to give a low blank, simply by passing it in the gaseous state through the double traps. This technique simplifies operation of the equipment and permits liquid air to be used when liquid nitrogen is not available. The liquefaction of oxygen permits a larger amount of the gas to be stored ready for use, but a simple calculation of the amount of oxygen consumed by the burning iron, silicon, etc., in the sample shows that it is adequately supplied by the volume of gas trapped by the mercury when the level of mercury is at  $L_3$ . The substitution of stopcock  $S_x$  for a mercury cutoff used in Wooten's apparatus has not raised the blank on the oxygen by any amount that will interfere with the determination. The grease used for this stopcock is Apiezon L.

**COMBUSTION SYSTEM.** The combustion chamber used for the earlier work permitted the insertion of only one sample into the system for each determination as shown in Figure 1. The sample dropped directly into the crucible before the side tube was sealed off. The disadvantages are, first, that air must be admitted to the system each time a determination is run; and the subsequent pumping and degassing are time-consuming. The air carries carbon dioxide and dust into the system, which may produce an increase in the blank. Secondly, the blank cannot be determined directly before the sample is run because of the presence of the iron sample in the crucible, and if air enters the system between the running of the blank and the running of the sample, it is not possible to ascertain whether the blank has been changed.

These difficulties were eliminated by the multiple loading system devised by Benjamin M. Walker of this laboratory, whose innovation speeded the operation of the method considerably and added to its precision. The diagrammatic arrangement of this loading tube is shown in Figure 2, and a photographic close-up is shown of one type of arrangement of the tubes holding the samples in Figure 4. The sample is held at  $a$  until a satisfactory blank has been obtained on the apparatus, and then is transferred by means of a magnet to the inclined glass tube, whence it is easily moved until it falls into the crucible. The number of such samples that can be sealed into the loading tube at any time is limited by the number that will fill the crucible, and this is dependent to some degree upon the type of sample used. The authors find that a crucible will take about 28 samples, and loading tubes hold 14 samples. The clinkers should not fill the crucible higher than to within 0.6 cm. (0.25 inch) of the top. When the crucible is thus filled it must be replaced by removing the top of the combustion vessel. In order to keep the equipment in continuous use, a duplicate combustion vessel with crucible and loading tubes with samples can be made ready and the complete combustion vessel units interchanged.

The platinum-iridium (0.4%) crucibles used were specially designed for the purpose by J. Bishop and Co., Malvern, Pa. They are of 15-ml. capacity and have welded to the rim a loop which allows the crucible to hang 9 cm. below the support in the combustion vessel. The platinum crucibles expand when heated, allowing the ceramic liner to slide down to a lower position which imposes a strain on the crucible when it cools to room temperature. The usual crucibles showed a tendency to crack around the upper rim, as well as about the bottom. The high-frequency current raises the point of such breaks to the melting point of platinum with disastrous results. By heavily reinforcing the bottom and the rim, this difficulty has been almost completely eliminated. At one time difficulty with high blanks was traced to a lot of crucibles which had apparently been contaminated by the formation of platinum carbide during manufacture. The blank could not be lowered by a cycle of repeated ignitions of the crucible in pure oxygen and pumping off to a low pressure, although the



process was repeated continuously for several days. Care should be exercised to get platinum free of carbon.

The first ceramic liner used for the platinum crucible consisted of a fused silica crucible, the bottom of which was covered with Alundum powder. The use of Alundum is objectionable because the blank it introduces and the space it occupies in the crucible. The silica, being acidic, is readily attacked by molten iron oxide, forming a slag which shatters the crucible and destroys its usefulness. G. H. Porter of the ceramic laboratory prepared ceramic liners high in magnesium oxide content. These proved very much more satisfactory and were eventually replaced by the magnesita liners described by Wooten and manufactured by Norton. Since that time Louise Harrop of this laboratory has prepared beryllium oxide crucibles which are even more satisfactory liners, by a method similar to that outlined by Thompson and Balliett (11). Dr. Harrop's liners are slightly smaller in outside dimensions, permitting a thin layer of powdered beryllia to be placed between liner and crucible. This arrangement greatly facilitates the removal of the liner from the platinum crucible after it has been filled with combusted samples. The blanks obtained on beryllia liners seem to be lower than can be obtained on the magnesita. It is expected that beryllia liners of this type may become commercially available.

**ANALYSIS SYSTEM.** The pumping system consists of a two-stage mercury diffusion pump of the type shown by Dushman (2) and manufactured by the General Electric Co. Details of the construction are shown in his article (2). The speed of this pump is considerably greater than of single-stage pumps that have been used. As backing pump for four units the authors use a Welch two-stage Duo-Seal vacuum pump, Catalog No. 1405H. The pump for the auxiliary vacuum used to evacuate the chamber above the mercury in the mercury wells needs to be capable of going to only a few millimeters' pressure.

Single traps are often too inefficient to trap condensed carbon dioxide. The solid material may be blown through the trap by a flow of uncondensed gas. Attempts to prevent this by using a plug of glass wool in the traps made it exceedingly difficult to get a good vacuum. Consequently, this technique was abandoned in favor of the double traps shown in the diagram.

The large hollow stopcock,  $S_7$ , with 15-mm. bore is vacuum-

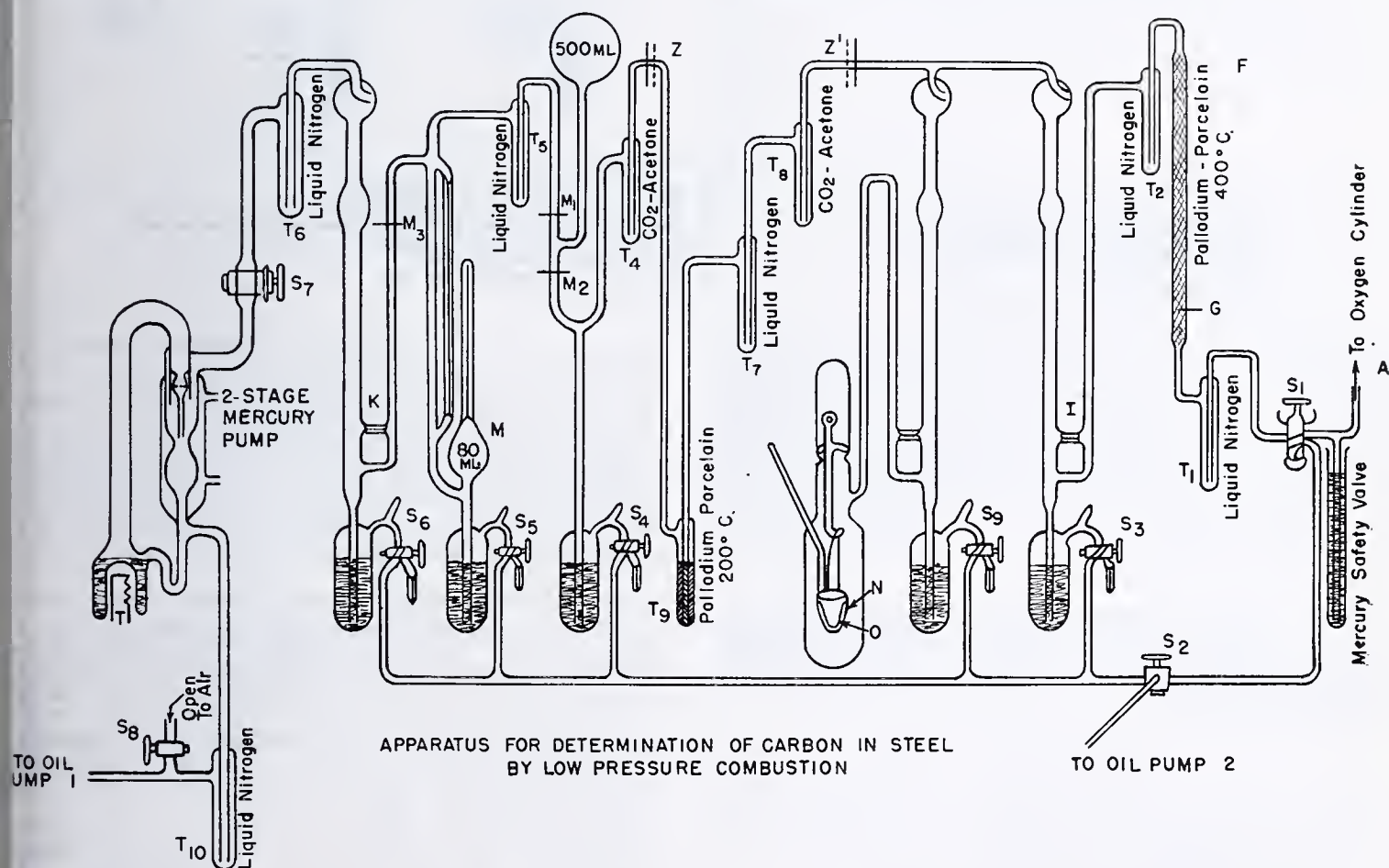
tight, of excellent construction, and can be obtained from Eck and Krebs, New York, N. Y.

The highest percentages of carbon reported by Wooten (13) are approximately 0.02%. The authors wished to analyze samples of steel containing as high as 0.06% carbon. It is not easy to get a representative sample of steel less than 0.5 gram nor is it convenient to work with samples much smaller than 0.5 gram; hence, the authors added an auxiliary volume (Figure 2,  $V' - V$ ) to their equipment, whose use is explained below.

They have used one 1.5-kva (output) Lepel Model C-3 high-frequency converter with a frequency range 170 to 500 kc. for four units. For more units they find it advisable to use more power and are now employing for eight units a General Electric 5-kva (output) power oscillator, Model 4F5A4, mean frequency 550 kc. The coil which surrounds the combustion vessel must be designed to conform with the characteristics of the oscillator. For each set of four units there is one coil. The two coils are connected in series and so to the oscillator. Air cooling must be provided to keep the walls of the combustion vessel from being overheated by radiation from the platinum crucible. The authors have used a small hand hair dryer clamped in a support or a jet of air from the laboratory compressed air system. An insulating casing, as of Herkolite, surrounding the high-frequency heater, is desirable to prevent the operator from actually touching the turns of the conductor. Although they are cold and a high-frequency current does not produce a shock, nevertheless an arc which will produce a deep burn can be drawn by accidental contact.

**CALIBRATING THE VOLUME.** In this method the volume of the McLeod gage must be known. First ascertain that the system has no leaks and earn the time required to evacuate the system from 5- to 10-mm. pressure to  $10^{-5}$  mm. (usually 10 to 15 minutes with mercury pump hot at the start).

Evacuate the system, holding the mercury down in both reservoirs. With pumps running, open  $S_8$  momentarily, admitting a small amount of air to the system. Quickly raise the mercury in the McLeod gage, thus trapping air in the bulb and capillary of the gage. Run the mercury up in the gage and determine the pressure of this trapped gas. Thus a known volume of gas is obtained,  $V_1$ , at a measured pressure,  $P_1$ . (The volume of the bulb



APPARATUS FOR DETERMINATION OF CARBON IN STEEL BY LOW PRESSURE COMBUSTION

Figure 1. Apparatus

F. Electric furnace at 400° C.  
G. Palladium black on porous Alundum  
M. McLeod gage  
 $M_1, M_2, M_3$ . Marks for confining calibrated volumes  
N. Platinum crucible  
O. Magnesita or beryllia lining crucible  
 $S_1$ . Mercury-sealed stopcock for admitting oxygen

$S_2, S_3, S_4, S_5, S_6, S_7, S_8, S_9$ . Precision-ground stopcocks  
T. Electric heater for mercury pump  
 $T_1, T_2, T_3, T_4, T_5, T_6, T_7, T_8, T_9, T_{10}$ . Liquid nitrogen traps  
 $T_4, T_8$ . Dry ice-acetone traps  
 $T_9$ . Trap filled with palladium black on porous Alundum  
Z, Z'. Extra equipment for separating  $H_2, H_2O, CO$ , and  $CO_2$



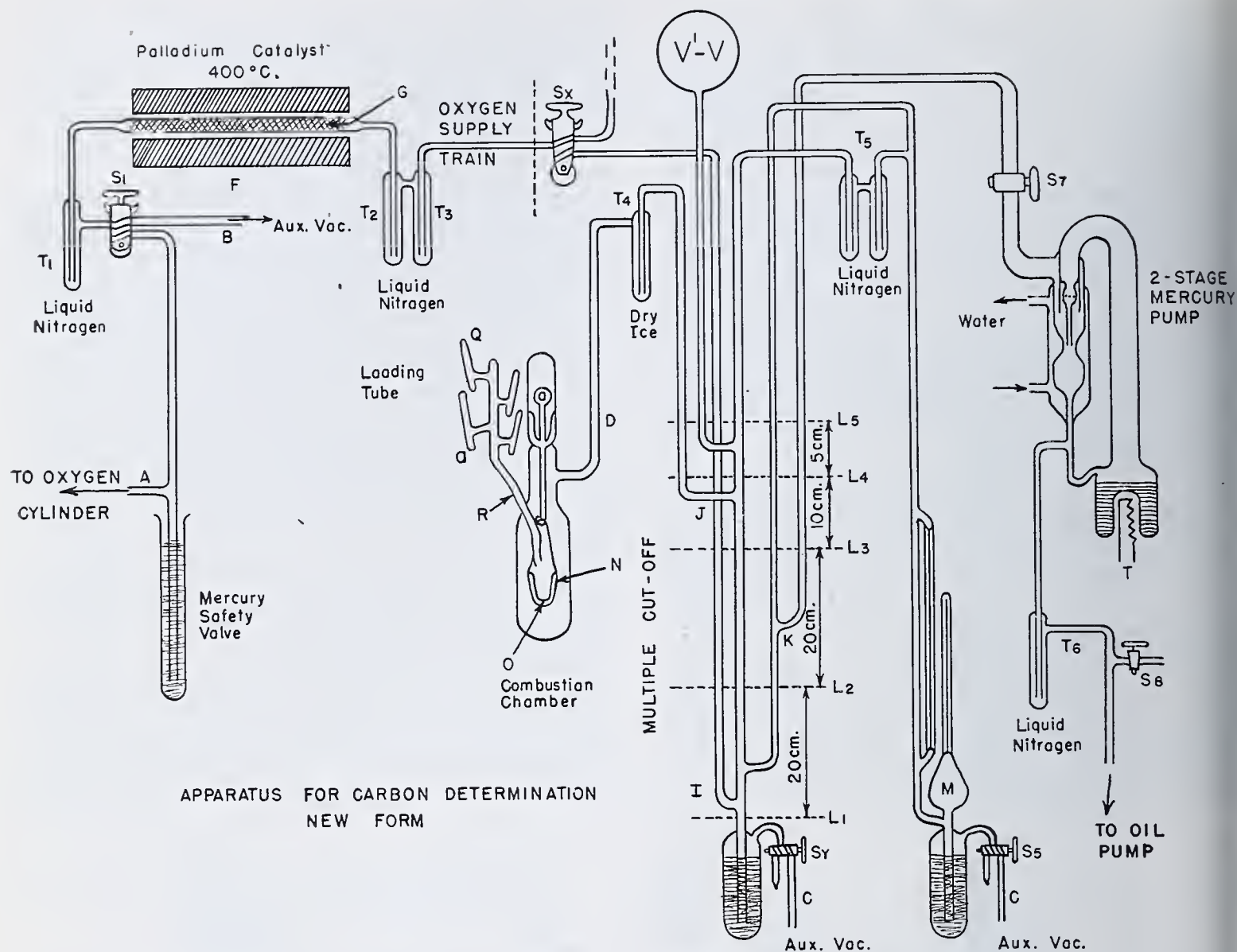


Figure 2. Apparatus

- a. Sample pocket  
 F. Electric furnace at 400° C.  
 G. Palladium black on porous Alundum  
 L<sub>1</sub>, L<sub>5</sub>. Marks for confining calibrated volumes  
 M. McLeod gage  
 N. Platinum crucible  
 O. Magnesia or beryllia lining crucible

- Q. Point of opening glass for loading samples  
 S<sub>1</sub>. Mercury-sealed stopcock for admitting oxygen  
 S<sub>2</sub>. Mercury-sealed stopcock for dividing oxygen supply to various units  
 S<sub>3</sub>, S<sub>4</sub>, S<sub>5</sub>, S<sub>6</sub>. Precision-ground stopcocks  
 T. Electric heater for mercury pump  
 T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub>, T<sub>5</sub>, T<sub>6</sub>. Liquid nitrogen traps  
 T<sub>4</sub>. Dry ice-acetone trap

and capillary of the gage were obtained during its calibration.) Leaving the mercury up in the gage to hold the trapped gas, evacuate the remainder of the system to  $10^{-5}$  mm. This must be done by timing, allowing more time for evacuation than the determined minimum. Raise the mercury in the multiple cutoff to L<sub>5</sub>, then lower the mercury in the gage and expand the trapped gas into the volume to be determined. Allow ample time for the gas to reach equilibrium pressure. Now measure the pressure,  $P_2$ , of the gas in the unknown volume,  $V_x$ .  $V_x$  may be calculated from the relation

$$P_1V_1 = P_2V_x$$

When  $P_2$  is measured, another volume of gas,  $V_1$ , is trapped at  $P_2$  and the system may be evacuated again and the gas expanded to  $P_3$ , etc. Thus a series of volume determinations may be made at decreasing pressures from the one original portion of air. The series of relations will be

$$\begin{aligned} P_1V_1 &= P_2V_x \\ P_2V_1 &= P_3V_x \\ P_3V_1 &= P_4V_x, \text{ etc.} \end{aligned}$$

in which  $V_1$  is the volume of the McLeod gage and  $V_x$  is the volume to be calibrated.

#### PROCEDURE FOR ANALYSIS

The preparation of the steel is the first important step in the analysis. The authors expected to have to reduce the steel to a finely divided powder in order to ensure complete combustion, but they were unable to do better than cut the silicon steel, usu-

ally in the form of sheets, into pieces weighing about 30 mg. All other methods resulted in contamination of the material from the carbon present in all the hardened tools they tried to use. Later experience proved that it was easier to convey heat to large steel fragments and the combustion of the steel was effected more easily and more thoroughly. Washing the sample with organic solvents was tried and eliminated after it was found that results on low-carbon samples so treated tended to be high.

**EVACUATING THE SYSTEM (Figure 2).** Close  $S_1$  and turn  $S_2$  so that the oxygen-purification system is connected with the analysis system. Close  $S_7$  and start the oil pump. Partially open  $S_7$  and begin to pump out the system from  $S_1$  to  $S_7$ . Keep the mercury down in the walls of the multiple cutoff and McLeod gage by cautiously connecting  $S_3$  and  $S_5$  to the auxiliary vacuum line. When the mercury ceases to rise from the two wells, open  $S_7$  wide and start the mercury pump. Evacuate the entire system until a pressure of  $10^{-5}$  mm. is reached. Keep trap  $T_4$  cooled with dry ice-acetone mixture at all times.

**ADMITTING OXYGEN.** Close  $S_7$ , leaving  $S_2$  turned to connect oxygen supply train to analysis system. Using the reducing valve on the oxygen tank, place a slight oxygen pressure on the line from A to  $S_1$  (this pressure will be indicated on the mercury safety valve). Raise the mercury in the multiple cutoff to L<sub>1</sub> by admitting air through  $S_3$ , and in the McLeod gage to the level of the cutoff arm of gage M by admitting air through  $S_5$ . By opening  $S_1$  slightly and maintaining a pressure from the tank, admit oxygen to the system until a pressure of about 15 to 20 cm



been attained. This pressure will be indicated by the mercury dropping back into the wells of the multiple cutoff and McLeod gage. The mercury safety valve serves to prevent excess pressure on the system from the tank, and also gives a visual indication of pressure which helps in adjusting  $S_1$  and the tank reducing valve while oxygen is being admitted. By constant adjustment of  $S_1$  and the tank reducing valve, the mercury can be kept level in the safety valve and gas admitted to the system without difficulty. When an oxygen pressure of 15 to 20 cm. has been attained in the system, raise the mercury in the multiple cutoff to  $L_3$  and connect  $S_1$  to the auxiliary vacuum. Pump out the excess oxygen until the mercury ceases to rise in tube  $I$ , then level  $S_1$  and  $S_2$ .

**TURNING THE SAMPLE.** By manipulation with a small Alnico magnet, move the sample through  $R$  into the crucible. Heat the aluminum crucible to 1200–1300° C. Direct a stream of air from a fan or compressed air line onto the combustion chamber to cool the glass. As soon as the crucible reaches maximum temperature, the sample will oxidize (if the silicon content is high enough it will burn), but the crucible is kept hot for 15 minutes longer to ensure complete combustion of the carbon. While the sample is burning, place liquid nitrogen around  $T_6$ . After combustion is complete, carefully open  $S_7$  just enough to raise the mercury in tube  $K$ , so that it is level with the mercury in the two tubes to the left of  $K$  (the difference in levels is caused by the oxygen used in the combustion). Lower the mercury in the multiple cutoff to  $L_2$  and open  $S_7$  slightly. This begins the removal of the excess oxygen while the carbon dioxide is frozen out in  $T_6$ . The mercury will rise slowly in the multiple cutoff, but must be kept below  $K$ . When the mercury ceases to rise, column will again be level with the others. Open  $S_7$  wide and pump the system until a pressure of  $10^{-5}$  mm. is reached. When the system is evacuated, raise the mercury to  $L_5$  and close  $S_7$ . Expand the carbon dioxide into  $V$  by removing the liquid nitrogen and allowing  $T_6$  to come to room temperature. Measure the pressure of the gas on the McLeod gage, or if the pressure is too high to read in the small volume, lower the mercury to  $L_4$  and take the pressure in the larger volume,  $V'$ . After having obtained the pressure of the carbon dioxide, lower the mercury to  $L_1$  and evacuate the system to a pressure of  $10^{-5}$  mm. before admitting oxygen for the next sample.

**CALCULATING THE CARBON CONTENT OF THE SAMPLE.** Substitution in the following formula will give the percentage of carbon in the original steel sample.

where  
 $V'$  = volume into which carbon dioxide expands  
 $h$  = difference in height of mercury levels in two capillaries of McLeod gage  
 $k_2$ , etc.) = constants for converting  $h$  to absolute pressure  
 There will be a different constant for each mark on the McLeod gage.  
 $T$  = absolute temperature  
 $w$  = weight of steel sample combusted

$$\text{Percentage of carbon} = \frac{100 \times 0.2729 \times 273 \times 44 \times V \times h \times k}{760 \times 22,400 \times w \times T}$$

Since some McLeod gages are calibrated directly in pressure, actual pressure may be substituted for  $(h \times k)$  which represents pressure in millimeters of mercury in this expression. Small changes in temperature do not affect the value of the expression appreciably. Therefore, for a fixed weight of sample there can be obtained for  $V$  (or  $V'$ ) a simple series of corrections for each calibration mark on the McLeod gage:

$$\text{Percentage of carbon} = \text{constant} \times h$$

This is easily converted to a graphical form for quick calculation.

**SENSITIVITY.** Since the difference in height of the mercury in the two capillaries of the McLeod gage is the final measure of the carbon in the steel, we may express the sensitivity of the method in terms of the height of mercury which corresponds to a given carbon content. When measuring carbon contents in the range 0.02 to 0.03%, using a 0.5-gram sample and expanding the carbon dioxide into the smaller volume,  $V$ , a difference of 10 mm. in the heights of the mercury columns at the highest reading of the McLeod gage (lowest sensitivity) corresponds to 0.00006% carbon. When reading the lowest pressures on the Mc-

Leod gage, a difference of 10 mm. corresponds to 0.00006% carbon. Obviously the sensitivity of the method increases with decreasing carbon contents. The inherent limitations which the authors have found in determining carbon arise not from the sensitivity of the measurement, but from the lack of uniformity in the distribution of carbon. The reproducibility found, working with samples in which the carbon content is seldom below 0.004%, permits fixing the carbon content within  $\pm 0.0005\%$ . For this reason the authors have been content to work with a larger blank than Wooten and Guldner. In general the blank averages 0.0005%. The blank has not been subtracted from any of the results reported in this paper.

When operations are started for the day, even when the apparatus has stood overnight under high vacuum, there is a high blank, usually 0.001% or higher. After running one sample of steel, the blank drops and, in general, continues low throughout the day. The authors can find no explanation for this phenomenon. Actual results are further discussed below.

**COMBUSTION OF SAMPLE.** The combustion of a steel sample of high silicon content will consume about 600 ml. of oxygen measured at 20° C. and 200 mm. pressure, assuming that the iron is oxidized to  $\text{Fe}_3\text{O}_4$  and the silicon to silica. Since the supply of oxygen is not in equilibrium with the liquid phase but is shut off from the supply before combustion starts, the pressure falls by about 70 to 80 mm. during the combustion. No difficulty seems to have been raised by this change in technique. The composition of the clinker remaining as a residue has been shown to be chiefly  $\text{Fe}_3\text{O}_4$  by the x-ray diffraction method.

In the earliest analyses, the sample after being introduced into the crucible was heated to about 500° C. for 5 minutes to drive off any adsorbed gases, particularly any that might contain carbon before oxygen was admitted. Results obtained from samples treated in this fashion were lower by about 0.001 to 0.01% than those run without this preliminary degassing, varying with the time of heating and the sample. A review of the literature (5) indicates that any such carbonaceous gases were derived from a reaction of carbon or carbides in the sample with oxides on the surface or in the steel.

In order to ascertain whether any carbon was given off in the form of monoxide when the sample was heated to 500° C., two traps and a catalyst were introduced into the system as shown from  $Z$  to  $Z'$  in Figure 1. The system was evacuated and the sample heated with the cutoff on the combustion vessel closed. After the sample had cooled, the cutoff was opened and oxygen admitted to the system. As the gases were pumped off, the water vapor was frozen out in  $T_8$ , the carbon dioxide in  $T_7$ . Any carbon monoxide and the residual oxygen passed over the palladium catalyst which was maintained near 200° C. by a boiling bath of trichlorobenzene. Any water from hydrogen which might have escaped previous combustion was frozen out in  $T_4$ . The carbon dioxide formed over the catalyst was frozen out in  $T_5$  and measured in the usual manner. This gas is allowed to escape and then by warming  $T_7$  the carbon dioxide previously condensed there is frozen out in  $T_5$  and measured.

The authors had no means for measuring the temperature of their sample, other than of estimating the temperature from the color of its incandescence. Carbon monoxide and carbon dioxide were both evolved; the proportion of the former increased with temperature in agreement with the results of Ryder (8). However, the composition of the gas also varied with the nature of the sample; pure iron appeared to give a higher proportion of carbon monoxide. Experiments conducted in a vacuum system of somewhat different design, provided with a Toepler pump to collect the gases evolved, indicated that at 1000° C. nearly all the carbon could be driven from a sample, provided the percentage of carbon was not too high. Samples of iron appear to have enough oxide on the surface or internally to bring about this oxidation. Since heating can remove an appreciable amount of carbon from the sample, the preliminary heating was dropped and the sample combusted without "degassing" the sample at an elevated temperature.



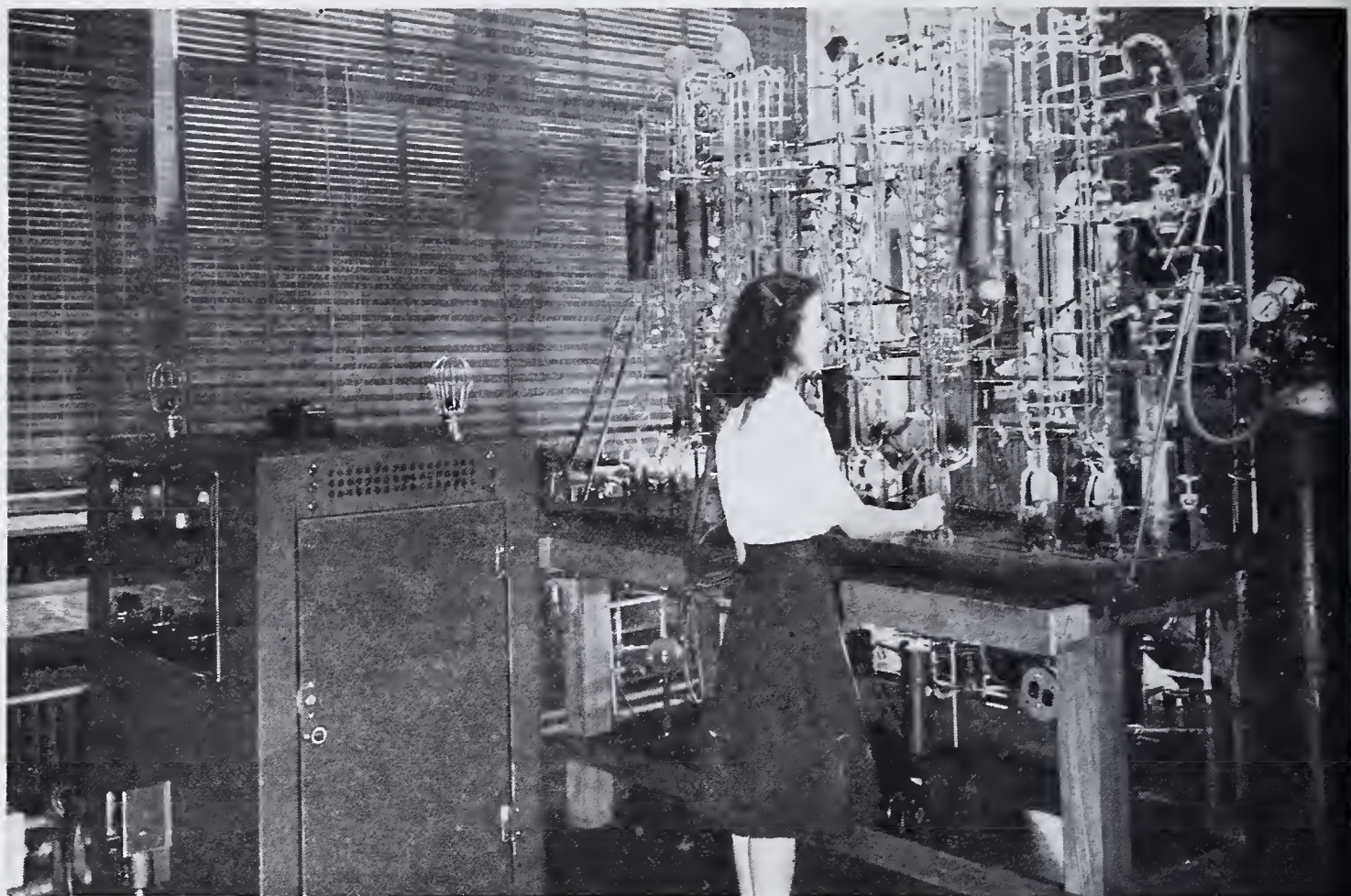


Figure 3. Low-Pressure Combustion Apparatus in Pittsfield Laboratory

No evidence of the incomplete oxidation of carbon to carbon dioxide when a sample is burned was found with the arrangement of apparatus in Figure 1.

When the platinum crucible is heated in the evacuated combustion vessel, no deposit is formed on the walls of the vessel. When oxygen is present, the wall becomes brown, then gradually black and opaque. The deposit, when scraped off and examined by testing its diffraction of x-rays, is shown to be platinum metal with some iron oxide ( $\text{Fe}_3\text{O}_4$ ). Since this deposit forms only when oxygen is in the system, the intermediate formation of platinum oxides seems not improbable (9).

#### RESULTS

In order to demonstrate the agreement that is possible with this method between various laboratories and different operators, the authors have compiled the data shown in Table I. They are indebted to J. B. Austin of the United States Steel Research Laboratory for the use of their data. Each "series" was run on one or more different apparatus or on apparatus completely dismantled and rebuilt. The data have been obtained over a fairly long period of time. The results grouped as "I Pittsfield G. E. (3 operators)" were accumulated over many months and on more than one apparatus. The operators included high-school graduates as well as chemists with postgraduate training.

The summary attempts to show how these six averages compare with the thirteen reported on the Bureau of Standards certificate for sample 55a. These results were obtained by selected cooperators who (with one exception) used the classical combustion method. After preliminary results are submitted by cooperators, the bureau customarily requests additional tests when reports deviate too grossly from the mean.

The results reported for the low-pressure combustion method represent all those obtained with the three exceptions noted. The elimination of the six values as indicated in Table I does not affect the mean of the six averages appreciably. The spread of the six

averages reported is 0.0014% (Table II) compared with 0.001% for the bureau's cooperators. The average deviation is  $\pm 0.0001$  compared with 0.001% (the fourth decimal place is not reported on the bureau certificate, hence this comparison is not strictly fair).

Table I. Summary of Results for Carbon Analysis

(Bureau of Standards Sample 55a)

Series	Reported by	No. of Determinations	Average, %	Average Deviation $\times 10^3$	Spread High-Low $\times 10^3$	Standard Deviation $\times 10^3$
1	Bell Telephone Laboratories (13)	10 <sup>a</sup>	0.0108	0.1	0.4	0.1
2	II Brackenridge G. E. (E. J. Fitz)	8	0.0109	0.4	1.1	0.3
3	I Brackenridge G. E. (E. J. Fitz)	11 <sup>b</sup>	0.0109	0.2	0.8	0.2
4	U. S. Steel Corp. (J. B. Austin)	10 <sup>b</sup>	0.0116	0.2	0.8	0.2
5	I Pittsfield G. E. (3 operators)	10 <sup>b</sup>	0.0121 <sup>c</sup>	0.3	1.3	0.3
6	II Pittsfield G. E. (L. Casali)	12	0.0122 <sup>c</sup>	0.4	1.9	0.3

Unweighted mean of six averages 0.0114

<sup>a</sup> Including additional value, 0.0110, sent by Wooten.

<sup>b</sup> After rejecting two values whose deviation from mean exceeds four times average deviation of rest of group.

<sup>c</sup> Averages in 5 and 6 on different samples of standard 55a.

Table II. Carbon Analysis

(Comparison of six averages of Table I with thirteen averages reported on B. of S. certificate 55a)

	% Carbon	Mean Deviation of Averages $\times 10^3$	Spread $\times 10^3$ (High-Low)	Value Reported
Recommended value B. of S. certificate	0.014	1.0	4.0	1.0
Mean of averages (Table I)	0.0114	0.6	1.4	0.6



Table III. Carbon in 3% Silicon Steel, Sample A			
Determined Carbon Value			
29 Determinations Run on Eight Units at Pittsfield (3 Operators)	21 Determinations Run on Four Units at Brackenridge (3 Operators)		
%	%	%	%
0.0034	0.0027	0.0027	0.0042
0.0029	0.0035	0.0023	0.0035
0.0027	0.0029	0.0036	0.0029 <sup>a</sup>
0.0027	0.0028	0.0027 <sup>a</sup>	0.0026 <sup>a</sup>
0.0032	0.0040	0.0027 <sup>a</sup>	0.0038 <sup>a</sup>
0.0034	0.0040	0.0030 <sup>a</sup>	0.0029 <sup>a</sup>
0.0026	0.0041	0.0038	0.0035
0.0029	0.0034	0.0033	0.0026
0.0028	0.0032	0.0026 <sup>a</sup>	0.0032
0.0031	0.0028	0.0033 <sup>a</sup>	0.0029 <sup>a</sup>
0.0033	0.0031	0.0042	
0.0031	0.0029		
0.0035	0.0027		
0.0033	0.0031		
0.0031			
Av. 0.0031		Av. 0.0032	
Standard deviation	±0.0003	±0.0005	
Standard deviation	±0.0004	±0.0005	
<sup>a</sup> High-carbon samples interspersed with these runs.			

Table IV. Carbon in 3% Silicon Steel, Sample B				
Standard combustion value, 0.006% carbon. Eight units, two operators.				
Date	Unit	Percentage Carbon		
		Blank (before)	Value	Blank (after)
Portion A, n = 11				
12-4-43	A	0.0010	0.0036	0.0006
12-4-43	C	0.0006	0.0046	0.0007
12-4-43	D	0.0006	0.0043	0.0006
12-6-43	A	0.0006	0.0040	0.0006
12-6-43	C	0.0007	0.0042	0.0011
12-7-43	D	0.0009	0.0038	0.0006
12-11-43	B	0.0005	0.0043	0.0006
12-14-43	C	0.0005	0.0041	0.0007
12-14-43	D	0.0008	0.0051	0.0009
12-15-43	B	0.0006	0.0044	0.0006
12-15-43	B	0.0006	0.0048	0.0007
			Av.	0.0044
			Standard deviation	±0.0004
Portion B, n = 9				
12-20-43	E	0.0009	0.0036	0.0004
12-23-43	E	0.0004	0.0050	0.0008
12-23-43	F	0.0005	0.0036	0.0005
12-23-43	G	0.0004	0.0042	0.0005
12-29-43	F	0.0004	0.0041	0.0006
12-29-43	G	0.0006	0.0041	0.0005
12-29-43	H	0.0007	0.0037	0.0006
1-8-44	B	0.0005	0.0042	0.0007
1-11-44	B	0.0005	0.0038	0.0007
General		av. 0.0006 <sub>s</sub>	General	av. 0.0006 <sub>s</sub>
		Av. 0.0040		
Standard deviation ±0.0004				

The most striking feature of the comparison is the fact that the average by the method under discussion is 0.011% compared with 0.014% reported by the bureau certificate. In general, it has been the authors' experience that their results by this method are a Bureau of Standards samples containing up to 0.3% carbon average a few thousandths of a per cent lower than the certificate values which are usually obtained by the classical combustion method. It is their opinion that occasional "wild" values obtained on even a carefully prepared well-mixed sample of steel come from a lack of uniformity in the distribution of carbon in the sample. It becomes evident with this method because of the small size of samples taken for chemical analysis.

Finally, series 2, 3, and 6 obtained by the authors' operators show somewhat poorer consistency than series 1 and 4. This probably reflects the effort to reduce the time required per determination and represents a slight sacrifice of accuracy in favor of speed. This is further discussed below.

In Tables III and IV are given typical sets of data to show the producibility that may be obtained with 3% silicon steel. All data over a given period of time are reported in both tables, with the exception of one very high value on portion A, Table IV. The precision of the authors' method seems to be consistent as measured by the standard deviation reported in Tables I, III, and IV.

Table V. Analysis of High-Manganese (14.4%) Iron Alloy			
Before Treatment		After Treatment	
Wt. of sample	Carbon, sample I	Wt. of sample	Carbon, sample II
Gram	%	Gram	%
0.05	0.0206	0.05	0.6191
0.1	0.0178	0.05	0.6502
0.2	0.0155	0.1	0.6780
Carbon Determined		Carbon by Weight Increase	
Sample II, % C after treatment	0.649	Sample II, % gain in weight	0.65
Sample I, % C before treatment	0.018	Sample II, % B added by treatment	0.02
Carbon added by treatment	0.631	Gain in weight corrected for B	0.63

A difficult question to settle in the determination of carbon is whether all the carbon is removed from the sample in the course of a combustion. In Table V are shown the results obtained in the recovery of carbon.

Two samples of high-manganese iron wire with weights shown were submitted by H. H. Uhlig of the G. E. Research Laboratory in Schenectady, who had observed a gain in weight under treatment which could be attributed to boron or carbon (lower right column). These data were not known to the authors at Pittsfield, and the carbon found by combustion was reported as given in the two upper columns. Boron was found in the wire, hence the gain in weight due to carbon is shown in the lower right column. This checks well with the authors' analysis. The spread in their carbon values in the upper two columns may be attributed to ununiform distribution of carbon as well as to the difficulty of completely burning the sample.

DISCUSSION

The time required for a determination by this method as described by Wooten and Guldner is not stated in their paper. The authors found that with the apparatus described, consisting of a single unit with one operator, a determination averaged about 2 hours, though under exceptional circumstances they have been able to average about one determination per hour through a single 8-hour day. A single operator can, however, manage about four units, provided he does not prepare and weigh the samples. With an assistant to prepare the samples, it is possible on the present equipment to average a single determination every half hour. A set of four units such as the authors have been using about 2 years is shown in Figure 3. Their experience has indicated that intelligent high school graduates can operate this equipment satisfactorily under the guidance of technically competent college graduates. When large amounts of data must be accumulated for statistical studies or production control, time and cost factors must be considered and will determine the feasibility of the analytical method.

The authors have attempted to adapt some of the older methods for carbon determinations. When the carbon and carbides are first separated by dissolving the steel in potassium copper chloride (7, p. 179) the amount of carbon recovered varied directly with the time the sample was centrifuged. Appar-



Figure 4. Combustion Chamber, Showing Multiple Loading System



Table VI. Carbon Content of Pure Copper

	Specimen A	Carbon	Specimen B
	%		%
Sample I	0.0017		0.0015
Sample II	0.0016		0.0025
Av.	0.0017		0.0020

ently some of the carbon separates in a form which tends to remain in suspension and is filtered off on sintered glass only with difficulty.

The barium hydroxide method (7, p. 172) has been tried, but with the type of absorber shown in Figure 39 (7, p. 173) it seemed impossible to get complete absorption of the carbon dioxide with any reasonable adjustment of oxygen flow, concentration of barium hydroxide, etc. (1). It is, therefore, necessary to use an empirical factor in calibrating the standard solution for this method, and the factor will change with any of a number of variable circumstances. The method does not recommend itself to the determination of small amounts of carbon. Attempts to improve the efficiency of absorption and to finish the analysis gravimetrically usually result in increasing the length of the method to an inconvenient degree (12). Good results were reported by Thanheiser and Dickens (10) using barium hydroxide as an absorbent for iron and steel containing a few thousandths of a per cent carbon. However, large samples of about 10 grams are required to secure the desirable accuracy, and, when the size of the sample is decreased, there is a correspondingly large decrease in the accuracy of the results.

#### CARBON IN COPPER

The only values given in the literature for the solubility of carbon in copper are those of Floe and Chipman (4). The authors were asked to determine the carbon content of a sample of

high-purity copper prepared in the laboratory by melting in graphite crucible under an atmosphere of nitrogen. When copper oxidizes, the heat of the reaction is less than for the oxidation of iron, and the copper does not burn. However, it is possible to oxidize it completely under the conditions described above for iron. The results on two samples of copper prepared as just described are shown in Table VI.

#### ACKNOWLEDGMENT

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## Determination of Carbon in Low-Carbon Steel

### Precision and Accuracy of the Low-Pressure Combustion Method

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The precision of the low-pressure combustion method described appears to be at least three times that of the standard combustion method when used on low-carbon steel. Its accuracy, as determined by direct calibration on Iceland spar, is about 0.0007% carbon, again about three times that of the standard combustion method.

THIS paper reports the results of a number of tests of the precision and accuracy of the low-pressure combustion method for carbon described by Wooten and Guldner (2). The apparatus and procedure are virtually identical with those of Wooten and Guldner, except that large single traps are used instead of smaller double ones, and during evacuation of the system after burning a sample the U-seal between the combustion and measuring section of the apparatus is closed at a pressure of 0.1 mm. of mercury, so that water may not be carried from the trap at dry ice temperature to the one in which carbon dioxide is condensed.

#### PRECISION

The precision attainable by this method is well illustrated by the analyses of National Bureau of Standards Sample 55a reported by Murray and Ashley (1, Table I). The authors have

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also investigated the precision relative to that of the common combustion method on four samples of steel, which proved to range in carbon content from 0.015 to 0.026%. These were submitted for analysis to four laboratories which use the ordinary combustion method, all accustomed to analyzing steels of this type, so that the results which each reports may be assumed to be reasonably representative of the precision that his procedure is capable of yielding. These results are given in Table I, columns A to D; the authors' results by the low-pressure method are in column E.

For each laboratory the mean for each sample was calculated and the deviation,  $\Delta$ , from this mean tabulated; for the sake of clarity these deviations have all been multiplied by 1000. The average deviation from the mean for all samples done by each laboratory was calculated from the arithmetic sum of the individual deviations—that is, without regard to the sign of each. This average is a measure of the precision of the analysis reported by each laboratory.

On the basis of these data, it is concluded that the precision of the low-pressure method is in general about  $\pm 0.0005\%$  carbon, although under favorable circumstances it may approach the value of the blank (about 0.0002), and that it is at least three times as good as that of the ordinary combustion method.



The authors' general experience with the low-pressure method, in which a 0.5-gram sample is used, leads them to believe that there may be actual differences in carbon content of samples nominally identical unless they are prepared and handled with meticulous care; consequently the precision of the result may be limited by that of the method than by the reproducibility, with respect to actual carbon content, of the sample analyzed.

### ACCURACY

The accuracy of a method of analysis can be gaged in three ways: (1) by comparison of the results with those of other methods; (2) by analysis of a standard sample whose composition has been determined by a number of different analysts, preferably using different methods; or (3) by analysis of a suitable pure compound, the composition of which is fixed and reproducible. All three methods have been used in the present case.

An indication of the accuracy of the low-pressure method relative to that of the common combustion method can be derived from Table I by comparing the results of the several analysts with a grand weighted average. In doing this, the question always arises as to who decides how the weighting is to be done. The authors have followed an impersonal procedure, frequently used, in which to each average is attached a weight inversely proportional to the average deviation from the mean of the individual results in which that mean was derived. Analyzing the data in Table I on this basis, they multiply for each sample the mean for each laboratory by the appropriate weighting factor (the reciprocal of the average deviation), and divide the sum of these products by the sum of the weighting factors (see 6.88). The resulting grand average is given here:

Average Deviation X 1000 (from Table I)	Weighting Factor	Grand Weighted Average for Samples			
		I	II	III	IV
0.8	1.25				
0.9	1.11				
1.4	0.71	0.0186	0.0151	0.0214	0.0261
2.1	0.48				
0.3	3.33				
	6.88				

The deviation of each laboratory's mean from the grand weighted average for each sample is shown in Table II. The mean deviation shown at the bottom of the column for each laboratory is derived by dividing the algebraic sum of the deviations for the four samples—that is, with consideration of the sign of each—by four. This mean deviation from the grand weighted average not only indicates the accuracy of a particular laboratory, but also shows whether that laboratory tends to report high or low values. On this basis, the low-pressure method has an accuracy twice to ten times that of the usual combustion method as carried out by the various laboratories.

As regards the second method of calibration, it was believed first that a Bureau of Standards sample would provide a suitable standard. Accordingly, the authors selected Sample 55a, whose certified carbon content of which is 0.014%, although the values reported by the laboratories which cooperated with the bureau in standardizing this sample ranged from 0.012 to 0.016%. Later they found that several other laboratories which use the low-pressure method likewise had been using this sample as a standard. The results of all these determinations are summarized by Murray and Ashley (1) and range from 0.0108 to 0.0122% with a mean of 0.0114, which is significantly lower than the certified value, though just at the lower limit of the range covered by the bureau's collaborators. The authors' general experience indicates that analyses made by the low-pressure method commonly yield a carbon content slightly lower than that obtained by the ordinary combustion method. The determinations

Table I. Comparison of Carbon Determinations

(By four laboratories and by the present procedure, on four samples of steel. The deviation,  $\Delta$ , in each case is the deviation  $\times 1000$  from the mean obtained for each sample by that laboratory.)

No. of Sample	Standard Procedure in Laboratory								Low-Pressure Method	
	A	B	C	D	E	A	B	C	D	E
I	0.019	0	0.016	-1	0.025	-1	0.028	+4	0.0165	-0.2
	0.018	-1	0.016	-1	0.020	-6	0.028	+4	0.0162	-0.5
	0.020	+1	0.014	-3	0.024	-2	0.027	+3	0.0173	+0.6
	0.034 <sup>a</sup>	...	0.020	+3	0.032	+6	0.015	-9	0.0171	+0.4
	0.031 <sup>a</sup>	...	0.018	+1	0.027	+1	0.024	0	0.0166	-0.1
	...	...	...	...	...	...	0.023	-1	0.0164	-0.3
Mean	0.019 <sub>0</sub>		0.016 <sub>8</sub>		0.025 <sub>6</sub>		0.024 <sub>2</sub>		0.0167	
II	0.011	-3	0.012	+1	0.011	-1	0.019	0	0.0170	-0.1
	0.014	0	0.012	+1	0.011	-1	0.017	-2	0.0169	-0.2
	0.015	+1	0.010	-1	0.011	-1	0.017	-2	0.0175	+0.4
	0.016	+2	0.011	0	0.013	+1	0.025	+6	0.0171	0
	0.013	-1	0.011	0	0.012	0	0.018	-1	0.0173	+0.2
	0.014	0	...	...	...	...	0.018	-1	0.0169	-0.2
Mean	0.013 <sub>8</sub>		0.011 <sub>2</sub>		0.011 <sub>6</sub>		0.019 <sub>0</sub>		0.0171	
III	0.020	0	0.024	-1	0.023	0	0.018	-2	0.0200	-0.5
	0.020	0	0.026	+1	0.022	-1	0.020	0	0.0199	-0.6
	0.019	-1	0.026	+1	0.023	0	0.021	+1	0.0217	+1.2
	0.019	-1	...	...	...	...	...	...	...	...
	0.020	0	...	...	...	...	...	...	...	...
	0.022	+2	...	...	...	...	...	...	...	...
Mean	0.020 <sub>0</sub>		0.025 <sub>3</sub>		0.022 <sub>7</sub>		0.019 <sub>7</sub>		0.0205	
IV	0.026	0	0.024	0	0.029	0	0.027	0	0.0260	0
	0.026	0	0.024	0	0.030	+1	0.027	0	0.0256	-0.4
	0.027	+1	0.024	0	0.029	0	0.028	+1	0.0264	+0.4
	0.027	+1	...	...	...	...	...	...	...	...
	0.027	+1	...	...	...	...	...	...	...	...
	0.026	0	...	...	...	...	...	...	...	...
Mean	0.026 <sub>8</sub>		0.024 <sub>0</sub>		0.029 <sub>3</sub>		0.027 <sub>3</sub>		0.0260	
Average deviation	0.8		0.9		1.4		2.1		0.3	
Maximum deviation	3		3		6		9		1.2	

<sup>a</sup> Not included because deviation is so much greater than in all other results of laboratory A.

Table II. Deviation (X 1000) of Weighted Mean from Average of Laboratory

Sample	A	B	C	D	E (Low-Pressure Method)
I	+0.4	-1.8	+7.0	+5.6	-1.9
II	-1.3	-3.9	-3.5	+3.9	+2.0
III	-1.4	+3.9	+1.3	-1.7	-0.9
IV	+0.4	-2.1	+3.2	+1.2	-0.1
Av. (% C)	-0.0005	-0.0010	+0.0020	+0.0022	-0.0002

of carbon in Sample 55a, instead of providing the desired check on the accuracy of the low-pressure method, have rather cast doubt upon the accuracy of the certificate value which should certainly now be reconsidered.

In view of the results on Sample 55a, special attention was paid to the third method of calibration, which was an absolute determination of the amount of carbon dioxide evolved from a crystal fragment of pure calcium carbonate (Iceland spar). A small crystal of analytical grade Iceland spar, carefully weighed on a microbalance, was placed in the magnesia crucible, oxygen was admitted, and the carbon dioxide determined by the standard procedure, special attention being paid to the rate of heating. The temperature of the crucible was raised from about 600° to 1100° C. over a period of about 20 minutes, after which it was maintained at this temperature for another 20 minutes before the carbon dioxide was collected.

The results of four such analyses (Table III) indicate that the average deviation of the observed amount of carbon dioxide from that calculated is  $0.03 \times 10^{-5}$  mole of carbon dioxide, equivalent to 0.0036 mg. of carbon, which for a 0.5-gram sample corresponds to 0.0007% carbon. On this basis, therefore, so long as the gas finally measured is substantially pure carbon dioxide, the accuracy of the method seems to be of the same order as its precision, since the average deviation shown in Table I is 0.0003% carbon.

### COMPOSITION OF GAS COLLECTED

Before one can claim for the analysis of a steel the accuracy indicated by the evolution of carbon dioxide from Iceland spar,



Table III. Absolute Standardization with Iceland Spar (Calcium Carbonate)

CaCO <sub>3</sub> Mg.	Weighed on	CO <sub>2</sub> Evolved (Pressure in Volume 251.9 Ml.) Mm.	Tem- pera- ture ° C.	Carbon Dioxide		
				Observed Mole	Calculated <sup>a</sup> Mole	Difference Mole
1.9	Analytical balance	1.460	30	$1.95 \times 10^{-5}$	$1.96 \times 10^{-5}$	$+0.05 \times 10^{-5}$
1.04 <sub>2</sub>	Microbalance	0.784	29	1.05	1.04	+0.01
1.25 <sub>6</sub>	Microbalance	0.918	28	1.23	1.25	-0.02
1.93 <sub>6</sub>	Microbalance	1.393	28	1.87	1.93	-0.06
Mean						0.03

<sup>a</sup> 0.5 gram of steel containing 0.05% carbon gives  $2.08 \times 10^{-5}$  mole CO<sub>2</sub>.

it is necessary to show that the material collected in the liquid nitrogen trap contains nothing other than carbon dioxide. That this is in fact true has been demonstrated by analysis of the condensed material by means of a fractional vaporization method based upon the fact that, as the temperature of the trap is slowly raised, there is for each condensed substance a characteristic temperature at which it vaporizes to cause a marked increase in the gas pressure of the system.

To carry out such a test, the trap was fitted with a copper shield, which helped to equalize the temperature, to which was attached a thermocouple. After combustion of a sample the excess oxygen was evacuated and the mercury levels were raised to isolate the calibrated volume. The trap was then quickly surrounded by an empty Dewar vessel which allowed it to warm only very slowly, and at suitable intervals the pressure was measured by means of the McLeod gage. For the materials condensed from the combustion of Bureau of Standards Sample 55a, which contained 0.02% sulfur and might have been expected to yield a little sulfur dioxide, the result of these measurements is shown by curve A, Figure 1.

The pressure, hence the number of moles of gas in the system, remained low up to about  $-120^{\circ}\text{C}$ ., at which temperature there was a relatively sudden increase to a pressure corresponding to the production of about  $4.5 \times 10^{-6}$  mole of gas; further increase of temperature up to  $0^{\circ}\text{C}$ . produced no further significant increase in the amount of material vaporized. The corresponding behavior of the condensate from the Iceland spar is shown by B, Figure 1. Had any sulfur dioxide or trioxide, or any other condensable substance, been present there would have been at a somewhat higher temperature another knee in these curves, as Wooten demonstrated by intentionally adding a little sulfur dioxide to the system.

Curve A indicates a slight evolution of gas between  $0^{\circ}\text{C}$ . and room temperature, an increase which is not evident in B. This increase, which was noted in all such runs on steel, indicates the evolution of less than 2% of some other gas, presumably water vapor. It is evident therefore that the gas condensed in the trap during combustion of a steel is virtually pure carbon dioxide.

The authors have also investigated the influence of two steps in the procedure upon the accuracy of the result. The first is the matter of washing the sample to remove superficial dirt and grease, which must be taken into account, since the surface of the sample is usually large and may therefore hold a relatively large quantity of carboniferous material.

If the sample is in the form of a thin strip, the surface can be cleaned by abrasion, after which the metal can be cut into small pieces with a pair of snips, avoiding any contact of the sample with the fingers or other material likely to contaminate it. If, however, the specimen is more massive, as, for instance, the fractured end of a tensile specimen, it is necessary to do some machining and in such case washing is desirable.

In the authors' procedure washing is carried out in a small Pyrex tube with a sintered crystalline Alundum bottom, the lower part of which is ground into a holder connected through a

stopcock to an evacuated filter flask. With the stopcock closed, a small amount of purified acetone is poured over the milling tube; the stopcock is then opened and the acetone slowly sucked away. After a minimum of three such washings the sample is placed in a vacuum desiccator and kept overnight at a pressure of about 1 mic (10<sup>-3</sup> mm. of mercury). In earlier work ether was used instead of acetone, but this was discontinued when it was found that humid weather evaporation of the ether chilled the metal enough to cause an unduly copious condensation of moisture. The question has also been raised as to whether some trace of acetone may not remain adsorbed or occluded on the sample, but direct comparison of the carbon content of a number

of washed samples with that of samples carefully cleaned but not washed indicates that when the washing is properly carried out the acetone remaining is not sufficient to increase significantly the carbon present.

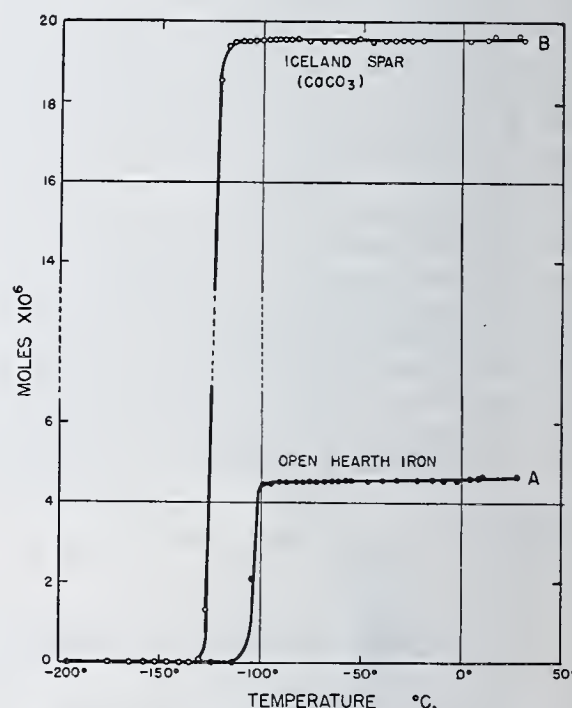


Figure 1. Fractional Vaporization of Condensed Carbon Dioxide

The other question considered is whether the true blank value—that is, with the high crucible temperature resulting from the burning of a sample—is appreciably different from that obtained in the normal way. To gain some information on this point a number of check runs were made on the same material after a preliminary heating of the crucible in oxygen and without opening the system, on the basis that if the higher temperature due to combustion of the sample did cause an increased blank, this blank should decrease on successive combustions, so that the apparent carbon content would successively decrease. The results indicated no significant trend in this direction.

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#### Photoelectric Photometer—Correction

In the caption of Figure 4 of the article "Photoelectric Photometer for Determining Carbon Disulfide in the Atmosphere" [*IND. ENG. CHEM., ANAL. ED.*, **15**, 593 (1943)] the caption should have read: R<sub>6</sub>. 250,000 ohms. R<sub>7</sub>. 15,000 ohms. R<sub>8</sub>. 50,000 ohms.

SHIRLEIGH SILVERMAN



# Measurement of Detergency

## A Photometer for Determination of Films on Transparent Surfaces

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A simple inexpensive photometer has been designed for the quantitative determination of "hard water" films formed on transparent glass plates during certain detergent processes such as commercial dishwashing. Constructional details and the electrical measuring circuit are discussed and information on the sensitivity of the instrument is presented.

THE calcium and magnesium salts present in hard water react with many detergents to form insoluble compounds. One of the precipitate thus formed attaches itself to objects being washed and in some processes, such as commercial dishwashing, builds up an unsightly film.

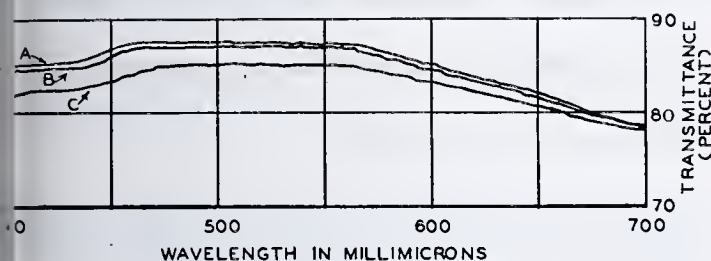


Figure 1. Transmission Data

One would expect the appearance of such film to be a function of such factors as the type of hardness and the amount present in the water used, the type and concentration of detergent used, the nature of the washing, rinsing, and drying processes, the temperature at which the processes are carried out, the nature of the surfaces being washed, the nature of the soil on these surfaces, and finally the number of times the objects are washed.

In commercial dishwashing, as ordinarily practiced, the build-up of unsanitary hard water or so-called lime films has presented a troublesome problem for the operators of public eating places. While progress has been made in recent years in the preparation of detergents which partially overcome this trouble, the relative importance of the various factors involved have not been known quantitatively, nor have satisfactory methods or instruments for use in the study of this problem been established.

This paper describes a photometer which has proved useful in obtaining accurate quantitative information regarding some of the factors involved in a study of this film build-up problem.

A relatively simple method now commonly used permits one to approximate roughly the comparative amounts of film produced when various detergents are used. Drinking glasses, glass plates, or other such objects are washed in a home-type dishwashing machine under known conditions, rinsed, and allowed to air-dry without toweling, and then the objects washed with different detergents are compared visually. Often several cycles of wash, rinse, and air-dry are required before good comparisons can be made. At best, however, such visual comparison yields only qualitative information which cannot be reduced to convenient numerical values for future use. An accurate and inexpensive instrument is therefore needed which can replace the visual comparison of objects washed and which will yield numerical quantitative data. Since these data are indicative of the visual

appearance of the film on the washed objects they have, for want of a better term, been designated as values of visual film thickness.

Since it is more difficult to make suitable optical measurements on drinking glasses or other irregularly shaped objects than on flat and regularly shaped ones, squares of plate glass were selected as test plates. Preliminary investigation indicated that the measurement of transmitted rather than reflected light is suitable for the purpose.

In order to determine whether a monochromatic light source would be necessary or of value in such an instrument, several sets of test plates washed with different detergent materials were prepared and the transmission spectra for the films on these plates were studied by means of a General Electric recording spectrophotometer. Transmission data thus obtained for the three most commonly used constituents of alkaline detergents are represented graphically in Figure 1. These studies indicated that the films are all essentially white—i.e., the amount of light transmitted is almost independent of the wave length used and wave lengths between 450 and 600 millimicrons are almost equally well transmitted. The deviations from "whiteness" are essentially independent of the detergent used. Therefore monochromatic light need not be used and any incandescent light source should be suitable with respect to wave length.

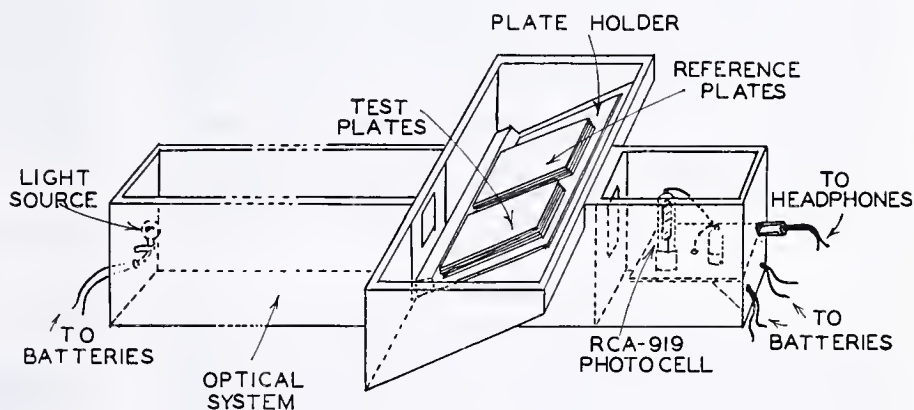


Figure 2. Photometer

An attempt was made to use commercially available photometers, but two construction characteristics rendered them unsuitable for this work. While a small beam of light is advantageous for most analytical work in that it permits the use of small volumes of solutions, it necessitates a large number of measurements if accurate averages are to be obtained on an object covered with an irregular film. Similarly, placing the photocell close to the absorption cell is advantageous in an instrument designed for work associated with colored solutions. For measurement of films on glass plates, however, usefulness is increased by placing the photocell farther from the plates on which measurements are being made. Such construction permits determination of beam transmission rather than diffuse transmission.







The "visual thickness" of the film on the test plates is then calculated by use of the formula:

$$\text{Visual thickness of film} = \frac{\text{apparent \% extinction}}{100\% - \text{apparent \% transmission}}$$

The washing and measuring procedure is then repeated the required number of times. For convenience each repetition of procedure is called a wash cycle.

#### REPRODUCIBILITY OF MEASUREMENTS

The reproducibility of measurements was determined by selecting a set of test plates which, after washing, had an apparent transmission of 57.0%. Over a period of 2 days this set of plates was measured ten times. After each measurement the plates were removed from the photometer and placed in a tightly covered box. The "reference-plates count" was intentionally varied from 78 to 100 clicks per minute. The probable error

was then calculated and found to be  $\pm 0.52\%$  for a single reading or  $\pm 0.17\%$  for the average of 10 readings.

Experience has shown that the errors resulting from the photometer are negligible as compared to those arising from variations in the wash procedure.

#### CONCLUSION

The photometer described is easily and cheaply constructed and possesses sufficient sensitivity to permit its use in the development of performance tests for certain types of detergents.

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PRESENTED in part before the Division of Industrial and Engineering Chemistry at the 104th Meeting of the AMERICAN CHEMICAL SOCIETY, Buffalo, N. Y.

## Measurement of Detergency. . .)

### Determination of Rate of Hard Water Film Formation in the Washing of Glass Objects

Method is outlined for measuring the tendency of precipitates formed by the reaction between detergents and calcium and magnesium salts to adhere to glass surfaces during washing. Data are

presented to show the reproducibility of results obtained by the suggested method and the ease and accuracy with which differences between detergents may be determined.

The relatively recent introduction of "polyphosphates" (1-4) has given the detergent chemist convenient materials which to decrease the rate at which film will appear on dishes and glassware when normal washing conditions are employed. However, there has been no satisfactory method of quantitatively measuring this rate of film formation. This paper reports a test capable of yielding reproducible results quantitatively evaluated by means of the photometer described in the previous paper (5).

#### METHOD

**WASHING MACHINE.** A home-type General Electric dishwashing machine has been found suitable to this type of test. Basically it consists of a covered tank, at the bottom of which is located a small impeller-type agitator. This agitator revolving at a speed of approximately 1700 r.p.m. throws the wash (or rinse) solution in the form of a fine spray over the objects which rest on a wire rack in the upper portion of the machine.

**TEST OBJECTS.** Pieces of plate glass 5 inches square and 0.125 inch thick cut from a single sheet of glass have been used as test objects. It is possible that the film build-up may be different on different types of glass and may vary with the physical as well as chemical nature of the surfaces of the test objects. Because the initial purpose of this work was to study the relative effects of different detergents rather than of different surfaces, these test objects were considered appropriate.

**PREPARATION OF STANDARD HARD WATER.** Water of known hardness may be obtained by filtering from some natural source enough water for the tests to be performed and then determining its calcium and magnesium content by analytical methods. A somewhat simpler method consists of softening a natural supply of water by means of a zeolite softener and then increasing its hardness by the addition of concentrated solutions of calcium and mag-

nesium salts. For this purpose the authors have used calcium chloride and magnesium sulfate; care must be taken in adding these salts to prevent the precipitation of calcium sulfate.

**ADDITION OF DETERGENT.** The desired weight of detergent for each cycle may be added directly to the wash water in the machine. Since the volume of wash water is small and the concentration of detergent is usually low, relatively small amounts of detergent are added at one time. Under these conditions a serious error may result, in that the composition of all additions may not be representative of the composition of the detergent being tested. This error may be partially nullified by adding a given volume of a concentrated solution of the detergent by means of an automatic pipet.

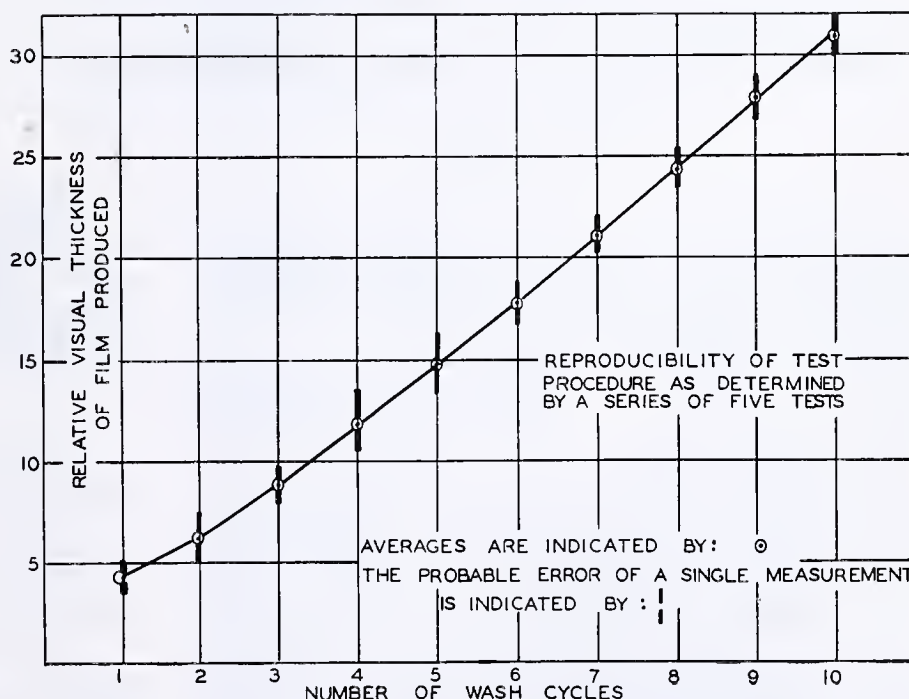


Figure 1



Table I. Reproducibility

(Detergent, trisodium phosphate. Concentration, 0.32%. Water hardness, Ca = 140 p.p.m., Mg = 50 p.p.m.)

Cycle	Apparent % Extinction						Probable Errors (in Terms of Apparent % Extinction)	
	1	2	3	4	5	Av.	r	R
1	3	4	5	5	5	4.4	0.56	0.25
2	6	8	5	6	8	6.2	1.20	0.54
3	10	9	7	9	10	9.0	0.82	0.37
4	13	10	10	12	15	12.0	1.42	0.63
5	16	16	12	13	18	15.0	1.60	0.72
6	20	19	16	16	19	18.0	1.26	0.56
7	23	22	20	20	22	21.4	0.82	0.37
8	25	25	22	24	27	24.6	1.22	0.54
9	29	31	26	27	28	28.2	1.35	0.60
10	31	32	29	31	33	31.2	0.95	0.42

Zeolite-softened water is heated to 160° F. by means of a thermostatically controlled water heater. A gallon of this water is drawn from the heater, hardened as previously described, and then poured into the machine. The temperature in the machine is indicated by a mercury-actuated dial-type thermometer whose bulb extends downward from the top of the machine. On starting the spray action the thermometer reading increases rapidly, until after about 15 seconds a maximum is reached and is recorded as wash temperature or rinse temperature. No decrease in temperatures has ever been noted during the 3-minute wash period.

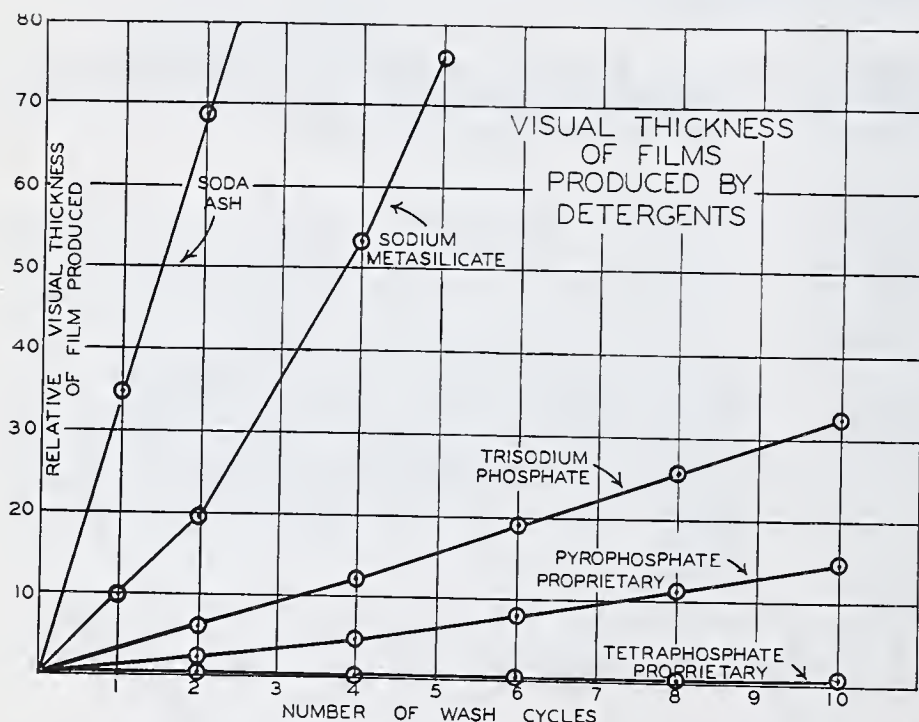


Figure 2

**SELECTED TEST CYCLE CONDITIONS.** Washing. Volume of wash solution, 1 gallon. Time of wash, 3 minutes. Temperature of wash water, 125° F. Hardness of wash water calculated as calcium carbonate, 140 p.p.m. of calcium and 50 p.p.m. of magnesium. Detergent concentration, 0.3%.

**Rinsing.** Immediately after washing, while still in the same position in the machine, the test objects were rinsed for 2 minutes at a temperature of 130° F., using water of the same hardness as that used in washing.

**Drying.** The test plates were dried by removing them from the dishwashing machine and placing them in a drying rack which held the plates at an angle of approximately 45° and separated one plate from another by about 0.5 inch. The plates were dried in approximately 1.5 minutes by a stream of warm air.

MEASUREMENTS of visual film thickness were made using the photometer described in a previous paper (5). In order to follow the regularity with which the film appeared on the glass test plates, measurements were made after cycles 1, 2, 4, 6, 8, and 10. Since the films formed by this wash procedure were not perfectly uniform—that is, since water spots or uneven films frequently appeared on the plates—measurements were made on two areas of

each test plate. This represented a measurement on 28 square inches of plate area. The instrument was standardized by making a measurement on the set of reference plates both before and after the measurements were made on the set of test plates.

## REPRODUCIBILITY OF RESULTS

The reproducibility of the results obtained by this test procedure is indicated by Table I. These data were obtained by repeating tests in which all controllable variables were maintained as nearly constant as was experimentally possible and using conditions outlined above.

The average probable error for a single reading was approximately 1.0% extinction. The significance of this probable error is best understood after studying Figure 1, in which visual film thickness has been plotted against the number of times the test plates were washed, rinsed, and dried. Because of the reproducibility of this type of test, differentiation between detergents which give nearly similar results is possible.

## DATA

Film build-up curves for three common constituents of commercial dishwashing compounds are shown in Figure 2. All data resulted from tests utilizing the conditions outlined above, the only variable being the composition of the detergent used.

Of the three alkaline materials most commonly found in commercial dishwashing detergents, commercial trisodium phosphate,  $\text{Na}_3\text{PO}_4 \cdot 12\text{H}_2\text{O}$ , produced the least film during ten repeated washing cycles. Sodium metasilicate,  $\text{Na}_2\text{SiO}_3 \cdot 5\text{H}_2\text{O}$ , produced a heavier film than the trisodium phosphate under the same test conditions but less film than soda ash,  $\text{Na}_2\text{CO}_3$ .

The use of certain molecularly dehydrated phosphates in reducing hard water film formation is illustrated by the two remaining curves. A notable improvement may be obtained by the introduction of tetrasodium pyrophosphate into the detergent. A proprietary containing 25% tetrasodium pyrophosphate, 40% sodium metasilicate, and 35% soda ash produced after 10 cycles a film having a relative visual thickness of only 13 units as compared to a thickness of 31 units produced by trisodium phosphate alone.

Greater effectiveness in preventing hard water films is possessed by other phosphates commonly referred to as polyphosphates. One of these, commercially designated as sodium tetrakisphosphate,  $\text{Na}_4\text{P}_4\text{O}_{13}$ , was selected for testing. Chemical analysis of this glassy material indicated a composition which may be

represented by  $3\text{Na}_2\text{O} \cdot 2\text{P}_2\text{O}_5$ . The results obtained with a proprietary containing 32% of this sodium tetrakisphosphate, 40% sodium metasilicate pentahydrate, and 28% of sodium carbonate show that such a detergent, under identical test conditions, yields a visual film thickness of less than 2 units.

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PRESENTED in part before the Division of Industrial and Engineering Chemistry at the 104th Meeting of the AMERICAN CHEMICAL SOCIETY, Buffalo, N. Y.



# A Compact Field Apparatus for Determination of Lewisite or Mustard Gas

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West Orange Gas Defense Laboratory, West Orange, N. J.

MEMBERS of the West Orange, N. J., Gas Officers' Defense Staff have developed a compact apparatus for field detection of lewisite or mustard gas (liquid). Its simplicity makes it suitable also for laboratory use.

When liquid lewisite is treated with 15% sodium hydroxide solution, acetylene is liberated and is detected in this apparatus by the formation of deep red cuprous acetylide on a disk of filter paper, freshly moistened with cuprous chloride solution. If the cuprous chloride solution is practically colorless, the moistened disk turns pink, then gradually deep red if considerable acetylene is generated; if the cuprous chloride solution is blue, the disk turns purplish red and the color gradually deepens. The reaction is distinctive, sensitive, and quantitative.

To test for liquid mustard, the paper disk is moistened with sodium platonic iodide-starch solution, without addition of sodium hydroxide or other reagent. In the presence of mustard the color changes from violet to strong blue. Gentle heat hastens the reaction.

## APPARATUS

The main tube, 1, has an expanded bottom bulb 3.75 cm. (1.5 inches) in diameter, on the side of which is blown a 2.5-cm. (1-inch) opening. A rubber stopper, 2, fits tightly in this opening and is provided with a 0.6-cm. (0.25-inch) hole in which is pushed tightly the end of a small vial containing the cuprous chloride reagent used to moisten the disk just prior to making the test

for lewisite. A rubber stopper is very satisfactory here, as neither lewisite nor mustard has any immediate effect upon it. After the test has been completed, the entire apparatus is taken apart and thoroughly scrubbed with calcium hypochlorite sludge.

Inner tube 3, centrally located in the upper part of tube 1, is supported in a cork having a small V cut out along its entire length, so that when cork and tube are in position, the gas evolved will vent through this cork and prevent the building up of pressure during the test. A slight bulb or bulge is blown 1.25 cm. (0.5 inch) from the lower extremity of this inner tube. Tube 4, connected with the upper end of tube 3 by 10 cm. (4 inches) of rubber tubing, has a capacity of 15 cc. Tube 4 has 1.5 grams of flake sodium hydroxide placed in it and is then tightly stoppered. Its contents will remain in perfect condition for any length of time. A small glass bead fits into the rubber tube, connecting tube 4 with the upper end of tube 3, about 2.5 cm. above the point where the rubber tube is fastened to tube 3. This bead, or valve, prevents liquid from entering tube 1. When the test is to be made, the side of the bead is pinched with the thumb and forefinger, causing a channel to be formed through which the caustic solution flows into tube 3, then into tube 1.

The filter paper disk upon which the color reactions are produced is cut to a diameter slightly smaller than that of the upper part of tube 1. In its center is cut a small hole of a size which will allow the disk to be pushed on the lower end of tube 3 up to the point where it meets the bulge. A small rubber band is twisted around the tube, flush with the underside of the paper disk, to keep it in position.

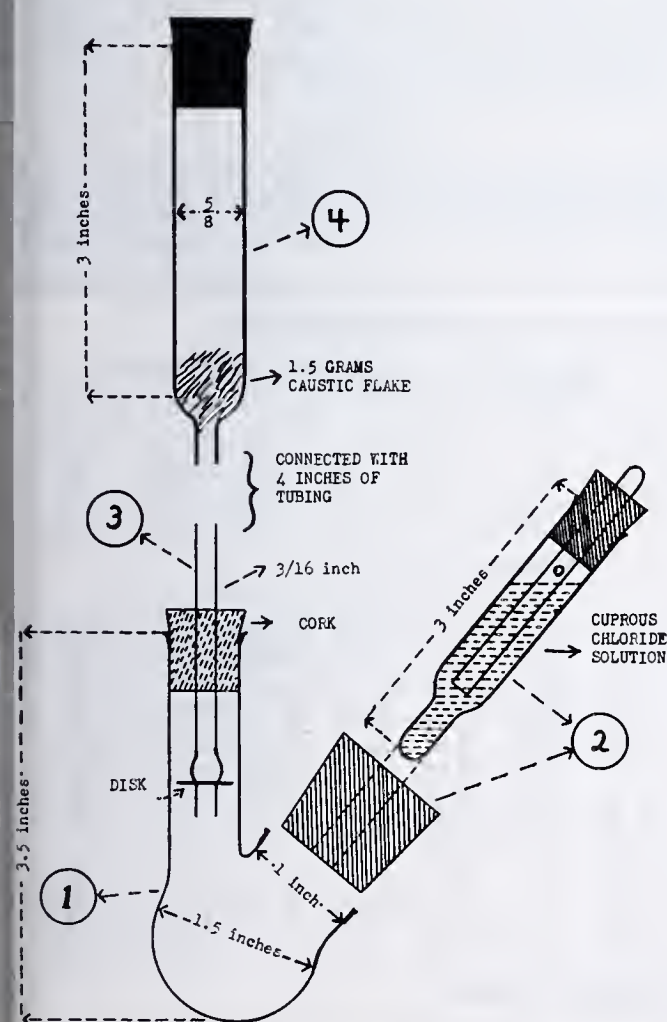
The supporting stand may consist of nothing more than a square block of wood containing a slight hollow in the center to support the bottom of tube 1 and an upright piece of wood with a clip near the top to support the upper end of the tube.

CONSTRUCTION OF VIAL AND CAPILLARY TUBE. The vial is made from soft-glass tubing of about 1.25-cm. (0.5-inch) diameter, the lower end being drawn down to fit the 0.6-cm. (0.25-inch) hole in the rubber stopper and sealed. The total length of the vial is 7.5 cm. (3 inches). The capillary tube, which acts as a dropper tube, inside the vial is 3-mm. glass tubing, open at the lower immersed end and having a very small hole blown in it just below the point where the lower end of the rubber stopper, which closes the vial, is located. The upper end of the capillary tube is sealed.

## PROCEDURE

Place the contaminated material, such as sand, leaves, rubble, twigs, etc., in tube 1 through the 2.5-cm. opening by means of tweezers or tongs, then firmly place rubber stopper 2 in this opening. Remove tube 3 with its dry paper disk, take out the capillary tube, and wet the disk with cuprous chloride solution by touching the open end of the capillary tube to the disk. Immediately replace the capillary tube and cork in the vial and return the inner tube with its moistened paper disk to tube 1. Remove the stopper from tube 4 and pour in 10 cc. of water, leaving the stopper out. Hold tube 4 straight above tube 3 and pinch the bead inside the rubber tubing, regulating the flow of solution, so that all the sodium hydroxide flake will have dissolved by the time all the water has run into tube 3 and thence into tube 1. (Considerable heat will be evolved, owing to the dissolving of the caustic flake.) The reaction of the caustic solution upon the lewisite is practically instantaneous and if any such "gas" is contained in the sample being examined, the filter paper disk will soon show its presence by a change of color.

Although the minimum sensitivity of this test when applied in the apparatus has not been determined, the West Orange gas laboratory staff has obtained very positive results with less than 0.25 drop of lewisite. The presence of arsenic may be confirmed by extracting the contaminated material, after the treatment with sodium hydroxide, with a small amount of water, neutralizing the solution thus obtained with 50% sulfuric acid, and then testing for arsenic by the Gutzeit test, using mercuric bromide paper and not mercuric chloride paper for the arsine color reaction.





ADVANTAGES OF APPARATUS

The chamber in which the acetylene or mustard vapors are brought into contact with the moistened reactor paper is very small, thus concentrating the amount of reactive gases in contact with the reagent.

The use of dry sodium hydroxide flake in tube 4 prevents deterioration and spilling of alkali.

The addition of the water to the dry sodium hydroxide just before use produces considerable heat, speeding the reaction. In the case of lewisite, this decreases the solubility of the acetylene in the liquid reactive mass and thereby increases to a maximum the amount of acetylene generated.

The filter paper disk, of practically the same area as the

chamber through which the reactive vapors must pass, gives quicker and better contact with such vapors than a paper suspended within the reaction chamber. The disk is placed at a point where contamination with the caustic solution is impossible.

The rounded bottom of tube 1 is an advantage if one desires heat the contaminated material gently (in case of mustard gas) for more rapid volatilization of the vapors.

Placing the cuprous chloride solution in the small vial, which turn is carried at all times in the rubber stopper, eliminates necessity of carrying a separate bottle for this reagent.

The entire apparatus may conveniently be carried in a pocket or field kit.

# Routine Determination of Zinc in Magnesium Alloys

## A Volumetric Method

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A rapid accurate volumetric method for the determination of zinc in magnesium alloys involves the precipitation of zinc in 1 *N* hydrochloric acid with excess standard potassium ferrocyanide. The excess is subsequently determined by titration with standard ceric sulfate solution. Manganese, cadmium, tin, and small amounts of iron do not interfere. The method is capable of an accuracy ranging from 1 to 5% of the amount of zinc present in magnesium-base alloys of high and low zinc content, respectively.

IN VIEW of the large number of magnesium-base alloys containing zinc, the determination of this element has become increasingly important to the magnesium industry. The Lang method (4) modified by Casto and Boyle (1) requires the elimination of manganese, cadmium, tin, and copper from the sample, and is limited largely to magnesium-base alloys containing aluminum and manganese. If the zinc content of the magnesium alloy is less than 1% a preliminary separation with hydrogen sulfide becomes necessary.

The method described in this paper is applicable to magnesium-base alloys containing from 0.05 to several per cent zinc, and is recommended for routine analytical control. Cadmium, tin, and manganese offer little interference. The error due to iron is largely compensated by a step in the procedure employing the use of potassium ferricyanide. If copper is present, it is removed with test lead.

### REAGENTS

POTASSIUM FERROCYANIDE, 0.025 *N* solution. Dissolve 11.2 grams of potassium ferrocyanide trihydrate analytical reagent grade, in 1 liter of distilled water containing 0.2 gram of sodium carbonate. Let the solu-

tion stand overnight, filter, and standardize against zinc chloride solution.

CERIC AMMONIUM SULFATE, 0.025 *N* solution (5). Dissolve 1.485 grams of ceric ammonium sulfate dihydrate in 1 liter of distilled water containing 28 ml. of concentrated sulfuric acid. The solution is approximately 0.025 *N*. Filter the solution, and adjust the volume so that 1 ml. of solution is equivalent to 1 ml. standard potassium ferrocyanide solution.

TRI-*o*-PHENANTHROLINE FERROUS SULFATE [(C<sub>12</sub>H<sub>8</sub>N<sub>2</sub>.H<sub>2</sub>O)FeSO<sub>4</sub>] solution. Dissolve 1.485 grams of *o*-phenanthroline (C<sub>12</sub>H<sub>8</sub>N<sub>2</sub>.H<sub>2</sub>O) in 100 ml. of 0.025 *M* aqueous solution of ferrous sulfate.

STANDARD ZINC SOLUTION. Dissolve 1 gram of zinc metal, c.p., in 20 ml. of 1 to 1 hydrochloric acid. Dilute to 1 liter.

Potassium ferricyanide, c.p., 0.5% solution. Concentrate hydrochloric acid, c.p. Dilute hydrochloric acid, 1 to 10. Test lead. Mercuric chloride, saturated solution.

### PROCEDURE

Weigh a 2.000-gram sample of the magnesium alloy into a 400 ml. beaker, and add 75 ml. of distilled water and 25 ml. of concentrated hydrochloric acid. Cover the beaker with a watch glass to avoid loss by mechanical spray. If copper is known to

Table I. Estimation of Zinc in Standard Chloride Solutions  
(In the presence of magnesium, manganese, aluminum, cadmium, tin, iron, and mercury)  
Milligrams of Metal Present<sup>a</sup>

	Mercury	Tin	Manganese	Cadmium	Aluminum	Iron	Magnesium	Zinc Taken	Milligrams of Zinc Found									
	...	...	...	...	...	...	...	Mg.										
10	9.70	9.52	9.83	10.37	9.57	10.70	9.83	10	9.70	9.52	9.83	10.37	9.57	10.70	9.83	10.01	9.57	9.86 <sup>b</sup>
10	9.55	9.88	10.01	10.42	9.60	10.83	10.09	10	9.55	9.88	10.01	10.42	9.60	10.83	10.09	10.29	9.52	10.75
10	9.91	9.80	9.98	10.44	9.96	10.55	9.96	10	9.91	9.80	9.98	10.44	9.96	10.55	9.96	9.93	9.78	10.62
Av. error	-0.28	-0.27	-0.06	+0.41	-0.29	+0.69	-0.04	Av. error	-0.28	-0.27	-0.06	+0.41	-0.29	+0.69	-0.04	+0.08	-0.38	+0.40
20	19.94	19.74	20.02	20.43	19.94	20.76	20.30	20	19.94	19.74	20.02	20.43	19.94	20.76	20.30	20.04	20.07	18.94
20	20.04	20.15	20.17	20.51	20.04	20.68	20.20	20	20.04	20.15	20.17	20.51	20.04	20.68	20.20	20.30	20.02	18.94
20	19.76	20.02	20.04	20.45	20.02	20.61	20.04	20	19.76	20.02	20.04	20.45	20.02	20.61	20.04	20.33	20.17	19.46
Av. error	-0.09	-0.03	+0.08	+0.46	+0.00	+0.68	+0.18	Av. error	-0.09	-0.03	+0.08	+0.46	+0.00	+0.68	+0.18	+0.22	+0.09	-0.65
50	50.00	49.64	50.25	50.43	49.38	50.76	50.23	50	50.00	49.64	50.25	50.43	49.38	50.76	50.23	50.18	50.12	49.92
50	49.82	49.69	50.48	50.66	49.72	50.66	49.56	50	49.82	49.69	50.48	50.66	49.72	50.66	49.56	49.74	49.74	49.81
50	49.84	49.79	50.15	50.76	49.59	50.59	50.12	50	49.84	49.79	50.15	50.76	49.59	50.59	50.12	49.82	50.07	50.18
Av. error	-0.11	-0.29	+0.24	+0.61	-0.44	+0.67	-0.03	Av. error	-0.11	-0.29	+0.24	+0.61	-0.44	+0.67	-0.03	-0.09	-0.02	+0.13
75	75.37	75.29	75.44	76.26	74.75	76.39	75.55	75	75.37	75.29	75.44	76.26	74.75	76.39	75.55	74.60	74.14	75.01
75	74.83	75.26	75.37	75.80	75.32	75.75	75.44	75	74.83	75.26	75.37	75.80	75.32	75.75	75.44	75.24	74.93	75.26
75	75.03	74.29	75.60	75.90	74.65	75.96	75.75	75	75.03	74.29	75.60	75.90	74.65	75.96	75.75	74.65	74.83	75.14
Av. error	+0.08	-0.03	+0.47	+0.69	-0.09	+1.03	+0.58	Av. error	+0.08	-0.03	+0.47	+0.69	-0.09	+1.03	+0.58	-0.19	-0.37	+0.13

<sup>a</sup> All determinations made in hydrochloric acid solution.

<sup>b</sup> Results in this column determined by potentiometric titration.

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Table II. Zinc Ferrocyanide Precipitates for Manganese Contamination\*

Manganese Added Mg.	Manganese Found in Precipitate Mg.	Zinc Added Mg.	Zinc Found Mg.	Zinc Error (Determined) Mg.
10	0.30	10	10.20	+0.20
30	0.58	10	10.60	+0.60
60	0.78	10	10.60	+0.60
10	0.30	20	20.60	+0.60
30	0.54	20	20.58	+0.58
60	0.78	20	20.60	+0.60
10	0.16	30	30.43	+0.43
30	0.60	30	30.78	+0.78
60	0.68	30	30.50	+0.50
10	0.30	40	40.80	+0.80
30	0.52	40	40.98	+0.98
60	0.68	40	41.00	+1.00

\* Each determination made in presence of 2 grams of magnesium metal as ferrocyanide.

Table III. Estimation of Very Low Zinc Content in Magnesium Alloy

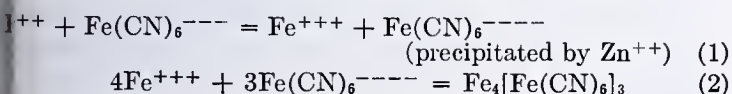
Polarographic %	Proposed Method %
0.065	0.07
0.067	0.07
0.065	0.07
0.065	0.07
0.095	0.10

present in the alloy, add approximately 3 grams of test lead to the sample, and boil for 5 minutes. Decant the solution through Whatman No. 1 filter paper into a 600-ml. beaker, wash the residue three times with 10-ml. portions of distilled water, cool to approximately 45° C., and add 2 ml. of potassium ferri-ferrocyanide reagent. Add a measured excess of standard potassium ferrocyanide reagent dropwise with rapid mechanical stirring. The dropwise technique should be observed for the first few milliliters, after which the reagent may be added more rapidly. Allow the precipitate to stand for 5 minutes, and filter by suction through 9-cm. Büchner funnel into a 500-ml. suction flask. The filter consists of a No. 42 Whatman paper covered with a thin layer of asbestos. Wash two times with 15-ml. portions of 1 to 10 hydrochloric acid. Titrate the excess potassium ferrocyanide reagent with standard ceric sulfate solution. Two drops of *o*-phenanthroline ferrous complex reagent are used as the internal redox indicator. An indicator correction of 0.2 ml. must be subtracted from the ceric sulfate titration.

If tin is present, add an excess of saturated mercuric chloride solution just prior to the addition of the potassium ferri-ferrocyanide reagent. Proceed as described above, making the final titration potentiometrically using a platinum indicator-saturated calomel half-cell electrode system.

# DISCUSSION

Iron (3) normally interferes with the precipitation of zinc as ferrocyanide, yielding high results. On solution of magnesium alloys in hydrochloric acid, the iron is present largely in the ferrous state. It appears that the addition of a 0.5% solution of potassium ferri-ferrocyanide reagent results in the oxidation of ferrous iron (2), producing at the same time an equivalent of ferrocyanide which is precipitated immediately by the zinc present. The ferric iron formed apparently remains in solution as a soluble ferric ferrocyanide complex. As the precipitation of zinc proceeds, the ferric iron present is precipitated as ferric ferrocyanide. It will be noted from Equations 1 and 2 that more ferrocyanide is produced through the addition of potassium ferri-ferrocyanide than can react stoichiometrically with the ferric iron formed:



If it is assumed that the above reactions go to completion, it would appear that 1 mole of ferrous iron produces 1 mole of ferrocyanide. The mole of ferric iron produced by the potassium ferri-ferrocyanide oxidation of ferrous iron will now react with 0.75 mole of ferrocyanide, which means that an excess of 0.25 mole of

ferrocyanide is produced. This yields low results for zinc, as illustrated in Table I. The iron normally encountered is much less than that added in this study. If care is not exercised in the filtration of a sample containing very low zinc and a few hundredths of a per cent of iron, a small quantity of colloidal Prussian or Turnbull's blue formed in the presence of excess potassium ferrocyanide may pass through the filter. This yields low results for zinc. The dropwise addition of standard ferrocyanide tends to reduce this potential error.

The acid concentration employed in this procedure is sufficiently high to avoid quantitative interference of such elements as cadmium, manganese, or tin. Cadmium alone does not particularly interfere. Cadmium and manganese together (or manganese alone) produce a slight deviation from true values. It will be noted from Table I that manganese causes high results. Table II illustrates the amount of manganese in a number of zinc ferrocyanide precipitates. The interference of stannous tin is overcome by addition of an excess of mercuric chloride prior to addition of potassium ferri-ferrocyanide reagent. Under these conditions, the end point with the redox indicator is indistinct; therefore, the titration must be carried out potentiometrically. Ammonium salts, excess sulfates, and nitrates interfere.

A single analysis on a magnesium alloy may be completed in 30 minutes. The method is capable of an accuracy ranging from 1 to 6% of the amount of zinc present on high and low magnesium-zinc alloys, respectively. On magnesium alloys containing less than 0.1% zinc the method is much less accurate (Table III).

In general, the method compares favorably with the accuracy attained by the hydrogen sulfide procedure for zinc and, in the hands of the average analyst, gives greater precision. Table IV illustrates the agreement between the two methods.

Segregation of zinc in magnesium alloys may account for some of the differences. Determinations were not made on aliquots. Therefore, Table IV shows the agreement in results which may be expected in ordinary routine analysis by the separate procedures.

Table IV. Estimation of Zinc in Magnesium Alloys

Hydrogen Sulfide Procedure %	Proposed Procedure %	Difference Based on Hydrogen Sulfide Method %
0.36	0.36	±0.00
0.21	0.20	-0.01
0.36	0.37	+0.01
0.18	0.18	±0.00
0.20	0.21	+0.01
0.52	0.51	-0.01
0.37	0.40	+0.03
0.42	0.43	+0.01
0.43	0.43	±0.00
0.48	0.48	±0.00
1.13	1.14	+0.01
3.09	3.15	+0.06
3.09	3.13	+0.04
3.14	3.14	±0.00
3.19	3.18	+0.01
3.24	3.26	+0.02
3.10	3.16	+0.06
3.14	3.17	+0.03
3.29	3.33	+0.04
3.04	3.10	+0.06

# ACKNOWLEDGMENT

The authors wish to acknowledge the fine work of John Mohan in establishing the accuracy of this method.

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# MICROCHEMICAL BALANCES

## Errors of the Kuhlmann Balance

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Methods are described for the location of many of the errors found in microchemical balances, particularly in the Kuhlmann balance. These fall into two general groups, those varying with the environment and those entirely resident in the instrument. In the first group are errors due to temperature gradients in the balance case, to humidity changes, to uniform temperature changes, and to magnetic influences. In the second group are those due to reading, to the imperfection of the knives, to poor arrestment design, to poor rider design, and to imperfect machining of the rider notches. Errors varying with environment can be controlled by the manufacturer by redesigning the instrument or by the user by controlling the environment. Under unfavorable conditions, they may range up to several hundred micrograms but under usual weighing conditions they are in the range of 0 to 20 micrograms. Random errors in the instrument are found to have a standard deviation of nearly 5 micrograms at full load. Methods are described for reducing the order of magnitude of these errors to about one microgram. Later communications will deal with other errors to be found in other balances and with details of design and construction which will eliminate these errors.

IT IS now generally recognized that with respect to speed and economy the methods of milligram analysis are superior to those of decigram analysis which have long been standard in organic analytical work. It is not so generally recognized that milligram methods as presently practiced are almost uniformly inferior in accuracy to the best decigram procedures which they are designed to replace. The accuracy of the determination of carbon and hydrogen on the milligram scale has been reported upon by Power (27). His findings contrast with the accuracy reported in decigram analyses by Benedict (2), Morse and Taylor (25), Barnett and Thorne (1), Bruun (3), Coffari (4), and Wing (39), to select only a few examples. In 1933 a program of research was initiated in this laboratory which aimed at discovering the fundamental reasons for the discrepancy in accuracy between milligram and decigram methods.

It was soon discovered that weighing is one of the most important sources of error in the milligram analysis for carbon and hydrogen. This alone is larger than the total error of most of the decigram methods referred to above. This finding has been substantiated by Corner and Hunter (6) and the Committee on Microchemical Balances of the AMERICAN CHEMICAL SOCIETY (32). When using milligram methods, the chemist is no longer secure in his accustomed assumption that the accuracy of his weighings is greater than the accuracy of his other manipulations. As a result of this finding, a systematic investigation to determine the causes of the irregularities observed was begun in this laboratory. This paper shows the presence and causes of weighing errors sufficient to bring about a maximum analytical error of 0.3% in carbon, assuming a 3-mg. sample containing 50% carbon with ideal laboratory conditions. With disturbing environmental conditions, this error may mount to 0.6% or even higher. Under normal conditions, the error would not frequently reach the maximum value.

Because the Kuhlmann balance is the most widely used among analysts, its performance was investigated first. In view of reports on the performance of the Kuhlmann balance in the literature and the results reported in this paper, it is amazing to find the statement by Pregl-Roth (28) that "this balance (the Kuhlmann) represents the limit of possible achievement in the construction of balances of precision". It seems certain that the

authors, in this instance, succumb to a common failing in fusing sensitivity with accuracy in balances. Thus they say, appears to be easily possible to obtain, with this instrument accuracy of  $\pm 0.001$  mg. on a weight of 20 mg.—that is, a sensitivity of  $10^{-7}$ ". The author has examined sixteen Kuhlmann balances in several laboratories and in no case was half an hour examination required to show that the accuracy of each instrument was only a fraction of its sensitivity. The studies recorded in this paper show that any analyst can make changes which improve the accuracy of his balance.

This communication presents test methods for locating and evaluating the errors of the Kuhlmann balance, many of which are also present on most other balances, and gives suggestions which will enable the owner of a balance to decrease the magnitude of many of the errors. Later papers in the series will attempt to assay all the features of design and workmanship in balances which can cause errors as large as 1 microgram.

In the course of these studies it was realized that no palliative measures would be sufficient to overcome the fundamental weaknesses in design of the Kuhlmann balance and that the only solution to the analyst's problem would be the construction of an entirely new balance with the errors of earlier designs eliminated. In the last few years, the importance of a domestic source of supply has become evident to all and this series of papers will present the results of numerous experiments on balance design, new and improved test methods, details of construction, tolerances, and specifications, which will permit domestic manufacturers to produce instruments superior to the best foreign instruments and with a reproducibility of 1 microgram under laboratory conditions. As a result of this study a new balance has been constructed in the author's laboratory which gives 1 microgram accuracy. Details of its construction will be given in the appropriate places in the series.

Examination of the data shows that the errors found may be divided into two categories: (1) errors caused by environmental changes and (2) errors entirely resident in the instrument. The latter fact has given rise to some confusion in specification and to variations in reports on balance performance.

### Errors Caused by Environmental Changes

Manufacturers possessing air-conditioned testing rooms with stable supports are frequently able to obtain better results with their instruments than users with laboratories less favorably equipped. Makers who are unable to guarantee that their instruments are so constructed as to be free from errors due to atmospheric and similar changes should know the magnitude of the effects to be expected and should be able to instruct prospective purchasers as to the best precautions to be taken under the available laboratory conditions. Their final objective should be to produce instruments which are not affected by the environmental changes encountered in a chemical balance room without air-conditioning or other special preparation.

Environmental changes which may affect balances and weighing are changes in temperature, humidity, barometric pressure, magnetic field, tilt of the building and balance supports, and vibration.

#### ERROR DUE TO TEMPERATURE GRADIENT

Manley (19) demonstrated with a differential bolometer that temperature differences exist within a balance case under the conditions of ordinary use and showed how the beam of the



balance could be protected against such an effect. In a later article (24) he ascribed zero point shifts to lengthening of the beam due to heating and to the change in buoyancy of air. Both effects should cause the pointer to shift as if the heated side of the beam were more heavily weighted.

In the summer of 1935 F. S. Arguelles of Seederer-Kohlbusch, Inc., called the author's attention to the fact that this could not be the true explanation, since it is easily demonstrated that the pointer actually shifts as if the heated side of the beam were less heavily weighted. This can be illustrated readily even with a relatively insensitive balance by placing a hot object above one of the pans. A rising current of air will result, which will cause an immediate illusory decrease in weight on the heated side. As far as the author is aware, no experimental method has been proposed heretofore for demonstrating whether or not the beam lengthens as Manley suggested (26). The results of such an experiment are reported here.

A study was made of the shift of the balance rest point under temperature differentials, with and without load. If the conditions causing motion of the air near the beam are reproduced, the apparent decrease in weight due to a rising air current will be the same at all loads. On the other hand, any effect due to an increase in the length of the beam will cause an apparent increase in weight proportional to the load transported away from the outer knife by the elongation of the beam. By varying the weight on the end knife, the effect due to the elongation of the beam may be differentiated from that due to the direct action of air currents. The experimental method by which this differentiation is accomplished is given below.

**EXPERIMENTS.** The following experiments cannot be performed with a Kuhlmann balance because of the impossibility of reproducing sufficiently the initial temperature differential in the Kuhlmann case. Instead, it is necessary to use a special experimental balance, heat-insulated to eliminate temperature gradients. The insulation process will be described in detail in a later communication.

A wire stand is arranged inside the balance case to support a heated object—for example, an empty absorption tube—in approximately the position which it would occupy if it were being weighed but so that no contact is actually made with the moving parts of the balance. Any weight desired may then be placed on the pans, the heated object inserted, and the excursion of the pointer noted at various times. The differences between the readings when loaded and those when unloaded at corresponding times, all other factors remaining constant, permit calculation of the effect due to changes in arm length of the beam. The absorption tube is placed in a 500-cc. glass cylinder in an air thermostat heated 5° to 10° C. above room temperature and allowed to remain until equilibrium has been established. It is then transferred inside the cylinder to the balance case and placed upon the wire stand as quickly as possible and the time is noted, preferably with a stop watch. Using this technique, satisfactory results may be obtained in plots of the displacement of the balance against the time elapsed after inserting the warmed tube.

The experiment is performed first with unloaded pans and then with a 20-gram load. The two runs are plotted, the weight of the pans and stirrups is determined, and a third curve is constructed which represents an extrapolation to zero load at the end knife. When the beam expands, it transports a portion of its own weight which cannot be calculated without knowing the temperature gradient in the beam from the outer knife to the end knife. The major portion of the load which it transports is upon the end knife, however, and for this reason we have chosen to neglect the weight of the expanding portion of the beam. With this inaccuracy, the extrapolated curve

represents the effect due to the direct action of the air stream alone, the difference between this and the actual curves being due to the expansion of the beam when heated by the air stream. For a further control, a thermocouple may be installed in the case with one junction as near as possible to the heated end of the beam and the other at the corresponding position near the unheated end of the beam.

The results of a typical experiment are given in Figure 1. An empty absorption tube was heated 9° above room temperature, 28° C., and temperature differentials between the end knives were read with a 40-gage copper-constantan thermocouple connected to a critically damped galvanometer showing a deflection of 1 mm. for 0.0004° C. In the first series of observations the pans were unloaded and zero point deflections and temperature differentials were recorded as nearly simultaneously as possible. These are plotted with white circles. In the second series of observations all conditions were the same except that the pans were loaded with 20 grams. The zero point deflections and temperature differentials are plotted with black circles. Both temperature differentials and rest point displacements are plotted as ordinates against time as the abscissa.

Examination of the curves in Figure 1 will show that an initial thermocouple deflection of 0.01° corresponds roughly to an error of 1 microgram but that the thermocouple equilibrates more rapidly than the beam. From this it may be concluded that temperature differentials as great as 0.01° between the ends of the beam are not to be tolerated. This is in general agreement with the calculations of Manley (18). The course of the thermocouple decrements in the two experiments serves as a control upon the accuracy of the reproduction of the conditions. In perfectly matched pairs of parallel experiments the two lines should be identical.

The curves show that after 200 seconds the air current produced by the slightly warmed absorption tube caused a shift of 63 micrograms in the rest point. Lengthening of the beam compensated this to the extent of about 10%, 7 micrograms, at zero load and more than a third, 23 micrograms, at full load. After 10 minutes the shift due to the air current had fallen to 21 micrograms, which was one-sixth compensated by the lengthening of the beam at zero load and 50% compensated at full load. Approximately 20 minutes were required for the heating error caused by the tube to disappear.

Obviously, the relative magnitudes of the air current effect and the expanding beam effect depend upon such factors as the size of the surface exposed to the rising air current, the presence of mechanisms which may act as baffles for the air stream, and the

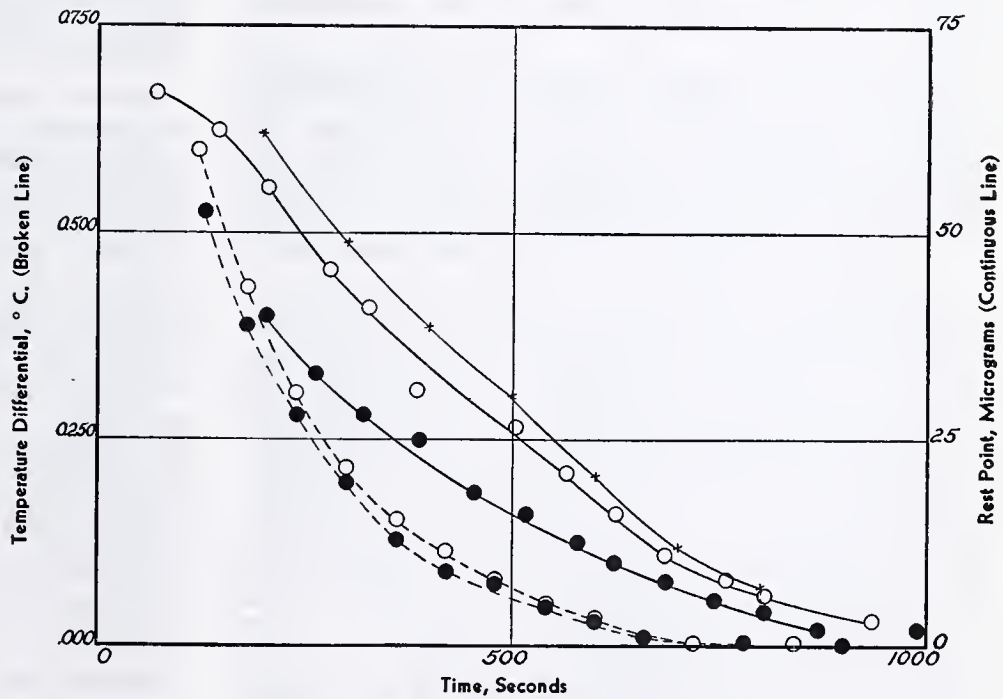


Figure 1. Effect of Warm Object on Balance

First series, unloaded pans: —○—○— rest point displacement; ---○--- temperature differential.  
Second series 20-gram load: —●—●— rest point displacement; ---●--- temperature differential.  
—x—x— rest point displacement extrapolated to zero load on knife.



thermal reflectivity of the beam. The author has been able to construct baffles so that the rising air current pushes the beam up from its tip but does not heat it appreciably, turns at the top, and gently descends to heat the arm on the opposite side. In this case the effects reinforce each other. Top and bottom compartments of the type recommended by Manley (20) are effective in preventing the heating of the beam but do not entirely remove the effects of temperature gradients on the zero point, since air currents in the lower compartment containing the pans can still affect the pans directly.

From the foregoing discussion, it can be seen that temperature gradients in the balance case can easily cause errors as large as 100 micrograms, thus necessitating the waiting period after loading which is recommended by Pregl. Further, even with a balance which does not permit a temperature gradient to arise from external heating in the laboratory atmosphere, the 5-minute period ordinarily recommended after a brief cooling might not be sufficient for complete temperature equilibration. With a poorly insulated balance, equilibration may never take place except momentarily by chance. Finally, the absolute magnitude of the effects due to gradients in temperature depends upon the details of construction of the balance but gradients as large as  $0.01^\circ$  are certainly to be avoided.

#### HUMIDITY COEFFICIENT

Changes in temperature ordinarily bring with them changes in relative humidity. Hence, if an instrument possessed a humidity coefficient, this would affect the results obtained upon exposing it to temperature changes. The importance of humidity control in weighing glass was recognized by Dumas and Regnault in early gas density determinations (7, 30). The weight of skins of moisture on glass was determined by Manley (21) and on metallic surfaces by Strömberg (36). Since laterally unsymmetrical effects due to humidity changes are conceivable, it is necessary to evaluate the humidity coefficient of a balance before it is possible to evaluate its temperature coefficient accurately.

If we assume that changes in weight of absorbing materials are linear with relative humidity, we shall have a convenient basis for comparison of results and for the setting of tolerances. Since it has been observed that atmospheric humidity may change as much as 10% per hour and since we wish no error as large as 1 microgram, we may set as an acceptable tolerance that a balance may shift 0.5 microgram with 10% change in humidity. This gives a tolerable "humidity coefficient" of 0.05 microgram per degree C.

**EXPERIMENTS.** A large "air-conditioned" box was constructed from composition board and covered with shellac inside. It was fitted so that the humidity could be increased by leading in air saturated with moisture or decreased by circulating the air over calcium chloride with a fan. As performed, the humidity tests were not strictly isothermal, variations as large as  $3^\circ$  C. taking place during the day or more required for a complete experiment.

A test balance constructed in the author's shops was used in this experiment and certain others. As is demonstrated in experiments recorded below, the only feature of importance in the humidity error is the size and nature of the bearings used. This test balance was constructed with large agate bearings the size of those used in commercial 200-gram capacity balances. The center knife weighed about 750 mg., the end knives about 275 mg. each, and end flats about 250 mg. each. The balance was placed in the box at  $25.5^\circ$  C. and 25% humidity, determined with a Friez precision hair hygrometer. The zero point was read and the humidity was then increased to 73%, the temperature changing meanwhile to  $28.3^\circ$ . The zero point shift was +142 micrograms, representing a humidity coefficient of 2.96. On drying, the rest point shifted in the opposite direction but did not return to its original value during the remainder of a working day, when the experiment was discontinued. It was difficult to secure reliable results by this method because of vibration.

The humidity coefficient may also be estimated by a more convenient method, requiring no special equipment except a hair hygrometer. The initial humidity and zero point are determined and then two sponges moistened with 5 to 10% sulfuric acid 2 to  $3^\circ$  above room temperature are placed in Petri dishes

or watch glasses arranged symmetrically with respect to the pans inside the balance case. The case is closed and the balance allowed to stand for at least 2 hours to equilibrate. Zero point readings are taken until no change is observed on 15 minute standing. The values of humidity and zero point are then recorded. Occasionally several hours are required for complete equilibration.

When the foregoing experiment was performed upon a Kamm balance, the zero point shifted 15 micrograms on going from 20 to 90% relative humidity. This is a remarkably small change but not a negligible one if accuracy to 1 microgram is essential since the coefficient is 0.214 or four times the tolerable coefficient chosen above. Drying the balance case caused the zero point to return slowly to its original value but 2 days were required before the process was complete.

It is possible to demonstrate that the humidity coefficient is due to the hygroscopic nature of the bearings, being almost entirely caused by the fact that water absorption by one end bearing is not exactly compensated by that of the other end bearing. This demonstration is accomplished by a series of experiments described below.

An ordinary agate center knife was cleaned with acetone and placed in a drying chamber under oil pump vacuum for 4 hours at  $100^\circ$  C., then cooled to balance temperature under vacuum and weighed as rapidly as possible. It was allowed to equilibrate with the atmosphere at 27% humidity and the gain in weight was found to be 603 micrograms. It is highly unlikely that the bearings made of this material would change by exactly equal amounts or at equal rates on humidification. Thus we have a possible explanation of the coefficient observed.

Two sets of end bearings, knives, and flats, selected at random from a large supply and identical in size with those used in the humidity test balance described above, were equilibrated again each other at 42% humidity and again at 83%. The exchange in weight over the change due to the balance itself was 152 micrograms. A balance with these knives would have had a coefficient of 3.71. This is comparable with that of 2.96 found in the test balance with similar bearings, showing that the observed humidity coefficient may be ascribed to unsymmetrical absorption of moisture by the end bearings.

It is not to be expected that any lack of symmetry in the respect in the center knife would seriously affect the result, since the average lever arm of the center knife is about  $1/60$  of that of the end bearings and the moment resulting from an unsymmetrical change in weight of the center knife would be correspondingly small. That this is true can be demonstrated by removing the end knives and bearings from a balance and measuring the humidity coefficient when adjusted as a compound pendulum. A balance in this condition was adjusted back to its original sensitivity by lowering the center of gravity nut and its humidity coefficient determined. Between 41 and 80% the shift in zero point corresponded to a change of only 2 micrograms, a coefficient of 0.051. Even this small change may be removed by the process of impregnation described below.

Since the humidity error may be of such magnitude, it was felt desirable to reduce or eliminate it before studying the temperature coefficient. One possible method for reducing the error is impregnation of the agate with a wax or plastic, the other search for a material of smaller intrinsic hygroscopic nature. Both these methods were tried successfully.

**Agate 1.** An ordinary center knife weighing 744 mg. was ground on all faces with 400-mesh Carborundum, cleaned, dried as above, and tested for hygroscopic gain as a control. On equilibrating at 30% humidity it gained 500 micrograms.

**Agate 2.** An ordinary center knife was refluxed in G. E. N. 3200 Bakelite "Monomer" (obtained through the courtesy of G. F. D'Alelio, head of the Plastics Laboratory, General Electric Co., Pittsfield, Mass.) for 24 hours, removed, and wiped. It was heated to  $70^\circ$  C. for 7 hours to remove alcohol and then cured at  $100^\circ$  C. for 24 hours. It was then ground on all faces with 400-mesh Carborundum to remove the resin on the surface. Finally it was dried as above and tested. On humidification it gained 50 micrograms. This experiment shows that it is feasible to force plastic materials into the pores of agate by this method.

The test balance used with plain agate in the earlier humidity experiment was fitted with knives and stirrup flat bearings impregnated as described for agate 2 and sponges were added to increase the humidity. Changing from 38 to 90% humidity



caused a shift of 72 micrograms. This is a coefficient of 1.38 compared with 2.96 found with the same balance with untreated knives. This represents an improvement over the earlier performance of the balance but not a satisfactory result. After repeated reimpregnation of these bearings it was found possible to reduce the humidity change to 5 micrograms with 50% gain in humidity, a coefficient of 0.10 or twice the tolerance. The process of impregnation improved the performance of this balance with respect to humidity changes over that of the Kuhlmann, even though the Kuhlmann has the advantage of much smaller bearings.

**Boron Carbide Flat.** This was a trapezoidal bar  $5-6 \times 6 \times 20$  mm. and weighing 686 mg. It was cleaned with acetone, dried, and tested as above. No gain in weight with humidification could be detected. Unfortunately, the test balance could be fitted with a boron carbide bearing only in the center. Up to the present time the author has not been able to fit a balance with boron carbide bearings throughout.

It is thus seen that the low porosity of boron carbide makes it an ideal material for removing the humidity coefficient due to microscopic knives but that more than 95% of the difficulty can be removed by impregnation with Bakelite, thus making a carefully impregnated agate bearing system available for use in the determination of temperature coefficients. A detailed discussion of the preparation and performance of these and other bearing materials will be given in a subsequent communication.

Since the determination of the humidity coefficient of an operating balance is such an easy experiment, requiring only a good dry hygrometer and the inconvenience of withdrawing the balance from use for a day, it is earnestly recommended that as many users as possible perform this experiment to acquaint themselves with an important feature of the performance of their instruments.

#### SHIFTS CAUSED BY UNIFORM TEMPERATURE CHANGES

A balance containing repeatedly impregnated agate bearings and fitted with an all-aluminum case as a shield was placed in an air thermostat. The zero point was determined and the air bath was heated to  $38-40^{\circ}\text{C}$ . and allowed to equilibrate at this temperature. The zero point was then redetermined. Numerous experiments of this character were performed and many troublesome irreversible effects were discovered which will be treated in greater detail in a subsequent communication. On the other hand, reversible shifts in zero point in a beam free from irreversible shifts and from humidity errors were found to be very small, of the order of 1 to 2 micrograms per  $10^{\circ}\text{C}$ .

Manley (24) speculates as to the cause of the observed temperature coefficients of balances and ascribes them to shifts in the relative positions of adjusting screws. It is easily demonstrated, however, that balances containing force-fitted knives have no temperature coefficients. In 1926 Manley (22) reported observations upon the behavior of a balance made with an Invar beam and agate knives. In spite of the supposedly small coefficient of expansion of the material of the beam, the balance still had a relatively large "temperature coefficient". All these observations support the conclusion drawn experimentally above that the major portion of the temperature coefficient of a balance may be in reality its humidity coefficient.

In an exchange of correspondence with W. H. F. Kuhlmann, manufacturer of the Kuhlmann balance, in 1935, the author alluded to the small temperature coefficient of the Kuhlmann balance. Dr. Kuhlmann replied that several users had confirmed the small temperature coefficient of his balances as contrasted to other balances but that he had observed that some of his balances had greater sensitivity to temperature changes than others. He said that the source of this discrepancy was a mystery to him and that he had been unable to discover its cause. It should be noted that Schwarz-Bergkampff (34) reported a relatively large temperature coefficient for his Kuhlmann balances.

An examination of the Kuhlmann balance shows that it has a much smaller weight of agate in the end knives and stirrup flat bearings than other balances. The end knife is truncated to insure a lateral adjustment of parallelism with the center knife

and the length and thickness of all end bearings are smaller than average. The flat bearings weigh less than 25 mg. and the knives weigh about 20 mg., making a total of about 45 mg. of agate in each end bearing as compared with 525 mg. in the test balance used above. Thus, if the humidity coefficients of both samples of agate were identical and the random match between the sets of end bearings equally good we should expect the Kuhlmann humidity coefficient to be 9% of 2.96 or 0.266 instead of 0.214 found. Better or poorer match between end bearings should give better or poorer coefficients, accounting for the observed variations.

These facts account for the remarkably small humidity coefficient actually observed on the Kuhlmann balance tested here and, in the opinion of the present author, they also account for the balance's small "temperature coefficient". The author's experiments with impregnation indicate that a single impregnation of the Kuhlmann agates should bring the humidity coefficient very close to the tolerance chosen. Thus three methods are open to the balance designer who wishes to produce an instrument with low humidity coefficient and low temperature coefficient: (1) use tiny agate bearings; (2) use more substantial agate bearings and impregnate them; (3) construct all bearings of a material like boron carbide which is insensitive to humidity changes.

Still another temperature effect should be pointed out in this section. The usual practice in balance construction is to make the beam from metal with a fairly high thermal coefficient of expansion and the knives from agate or other material of low coefficient. When a beam so constructed is subjected to extreme temperature changes, strains may be set up which will permanently affect the adjustment of the knives. Thus a Kuhlmann balance was exposed to an outside temperature of  $-21^{\circ}\text{C}$ . overnight and when it was allowed to regain room temperature it was found that the sensitivity-with-load relationship was permanently changed, the sensitivity at full load being only 60 instead of 100 micrograms. This shows that it is not desirable to ship precision balances during extremely cold weather.

#### MAGNETIC INFLUENCES

The possibility that variations in the earth's magnetic field might influence the zero point of a balance was apparently first appreciated by Manley (21), whose Invar beam was ferromagnetic. An appreciable error due to the use of steel bearings was also found by McBain and Tanner (15) in a more sensitive balance. The use of magnetic material in the moving parts of a precision balance is to be deplored, yet the Kuhlmann balance has steel screws that are used for adjusting the positions of the knives. Since magnetic field changes in a modern laboratory may be considerably greater than those resulting from the diurnal variations in terrestrial magnetism which affected Manley's instrument, it was deemed necessary to determine the magnitude of the effect of magnetic field variations upon the Kuhlmann balance.

**EXPERIMENTS.** A tangent galvanometer was modified by the removal of its base and its indicating needle in such a fashion that it could be used as the source of a small magnetic field of known intensity. The large coil was supported in a horizontal position on top of the case of the Kuhlmann balance and readings were taken without the current flowing and immediately afterwards with the current flowing but without arresting the beam. Duplication of the experiment gave results concordant within the reading error of the instrument. Imposing in this manner a vertical field of 1.7 c.g.s. units caused a zero point shift of 10 micrograms. Since the vertical component of the magnetic field at the laboratory was 0.552 unit at the time, the deviation from the true rest point caused by the vertical magnetic component was 3.3 micrograms. J. A. Fleming, director of the Department of Terrestrial Magnetism, Carnegie Institution of Washington, has very kindly informed the author that during times of great magnetic disturbances variations as large as 0.02 c.g.s. unit are found in Washington. Using this figure, we should calculate a maximum disturbance of 4% of the total force or 0.1 microgram, due to variations in the vertical component.



The experiment was repeated with the coil of the tangent galvanometer encircling the case of the Kuhlmann balance at the center and in a vertical position, thus creating a horizontal field parallel to the beam. Imposing a field of 2.28 units caused a change of 69 micrograms. Since the horizontal component of the field at the laboratory was 0.207 unit at the time, the permanent deviation from the true rest point caused by this field was 6.5 micrograms, corresponding to a maximum variation of 4% of this amount or 0.24 microgram.

It is thus apparent that the degree and distribution of residual magnetism in this particular beam are such that the errors caused by the earth's magnetic fluctuations are smaller than 1 microgram. On the other hand, magnetic fluctuations caused by electrical installations such as motors, generators, rheostats, or resistance furnaces may be many times those of the earth. An analyst using a Kuhlmann balance or any other with magnetic parts in the vicinity of electrical installations would have to take precautions to ensure protection against this effect. This could probably be most easily achieved by the use of a soft iron screen of high permeability and low retentivity.

The magnetic error of the Kuhlmann balance is small in a vertical field because the magnetic screws are symmetrically placed with respect to the knife in the horizontal direction. Thus vertical fields bring about only small torques due to differences between the screws with respect to residual magnetism. The relatively large sensitivity to horizontal fields is caused by the fact that in the vertical direction the screws are unsymmetrically placed, exerting a considerably greater torque above the knife than below it. Occasionally, analytical balances have been found with magnetic pointers. These would cause more serious errors due to the large moment which would be exerted by a force at such a distance from the knife edge.

#### OTHER ENVIRONMENTAL EFFECTS

Changes in barometric pressure can affect results only if the materials of the two sides of the balance are of different density. Such differences are seldom observed in balances themselves but are regularly found in microchemical weighings, due to faulty taring. Taring by the Pregl method can give rise to errors as large as 100 micrograms or more in a single absorption tube if the barometric pressure changes by 20 mm., a change which has been observed in this laboratory on more than one occasion during storms. The importance of proper taring has been fully understood by chemists for many years and satisfactory methods were developed by Regnault (30) in 1845 to permit the determination of gas densities. Discussions of the applications to microchemical weighings can be found in articles by Friedrich (8), Williams (38), and MacNevin and Varner (16).

The effect of tilting of the balance support was pointed out by Manley (24), while vibration has been discussed by Kirner (13) and Howard (12). Further comments on these subjects will be made in a later communication.

#### Errors Entirely Resident in the Instrument

The instrumental errors which are independent of environmental changes fall into two classes. Most of them are random errors which may be treated by statistical methods. These have been applied by Corner and Hunter (6) and the methods used in the author's laboratory do not differ essentially. Two do not fall into this category and will be discussed separately.

**DATA AND STATISTICAL METHOD USED.** The primary objective of the statistical procedures followed in recording the observations below is not to evaluate errors accurately but to locate them and to make possible their reduction or elimination.

In the accompanying tables are recorded the number of observations, the standard deviation,  $\sigma$ , and another measure of reliability which seems appropriate for the purposes of the investigation, designated as  $E_{99}$ , defined as  $E_{99} = 2.65 \sigma$ . If we assume a normal Gaussian distribution of errors, the probability is that 99% of the errors are less than  $E_{99}$  and only 1% is greater.

Since many of the errors studied approach normal distribution fairly closely, a deviation of the magnitude of  $E_{99}$  will encompass practically all the valid observations. This measure is adopted because of the custom frequently followed by analysts recommended in standard works on microchemical procedure permitting the balance to stand for a predetermined time after closing the case, then releasing the mechanism and making a single observation of the deflection from three readings of pointer. Assuming that there is no drift in the balance but that only random errors are at work, what is the maximum error to be expected from this procedure? The answer to this question is the real test of the validity of the procedure.

While the criterion,  $E_{99}$ , might appear to be unreasonable rigorous, it should be remembered that the determination of carbon and hydrogen requires six weighings and so, on an average, one out of seventeen analyses will be affected with an error of this magnitude or greater. On the other hand, the chances that errors of this magnitude will affect more than one weighing in an analysis are negligible.

Standard deviations have been calculated by the use of the formula

$$\sigma = \pm \sqrt{\Sigma(v^2)/(n-1)}$$

where  $v$  is the variation of each observation from the mean and  $n$  is the number of observations.

Many of the sets of observations recorded have been found to approximate roughly a Gaussian curve and others to represent a curve only slightly skewed. It follows immediately that the simplest method for increasing the accuracy of results in microchemical analysis is to adopt the time-honored device of making a large number of observations than one and to use the arithmetic mean. The observations recorded in the following sections give an idea of the efficacy of the statistical method in improving the precision of the weighings in various circumstances and of the limitations of the method as applied to balances commercially available.

Table I. Reading Error

No. of Observations	$\sigma$ , $\gamma$	$E_{99}$ , $\gamma$
14	0.8	2.1
10	1.3	3.4
10	0.8	2.1
10	0.9	2.3
10	0.8	2.1

#### READING ERROR

It is obvious that the process of interpolation commonly employed in estimating the pointer reading to 1 microgram on commercial balances will cause an error whose magnitude will vary with the operator and with the conditions of the observation. To dissociate this error from all others is very difficult, yet an estimation of its magnitude must be obtained in order to evaluate other errors.

The method adopted is to release the balance so that it has a moderate amplitude of oscillation and to record a series of ten more successive deflection determinations, each calculated as usual from three reversal points. If the ten show a marked drift, the error can usually be laid to unequal heating, as has been developed above. In this case the set is discarded for the purpose of evaluation of the interpolation error. If a sharp break is recorded in the series of deflection determinations, that the values fall into two distinct series, the members of which agree closely, the error at the break is probably due to mechanical damage at the knife, as is developed below. In this case also the set is discarded. If neither drift nor break intervenes, it may be assumed that the residual errors are due to interpolation. A series of ten is probably not sufficient to estimate the error accurately, yet a series very much longer is difficult to procure owing to decrement. Table I gives the results of five series of observations determined by different observers in this laboratory on a Kuhlmann balance equipped with a concave mirror. Each observation is calculated from three reversal points.



According to these results, the observer who made the second series would not be justified in relying on a single interpolation as being more accurate than 3 to 4 micrograms. For general purposes we may assume that the  $E_{99}$  due to reading error is of the order of 2 micrograms. It follows immediately that the operator who wishes to assure microgram accuracy must provide himself with a balance that reads directly to micrograms and automatically eliminates the interpolation error. If such an instrument is not available, he must have recourse to multiple observations. The accuracy of the observations on the Kuhlmann concave mirror balance

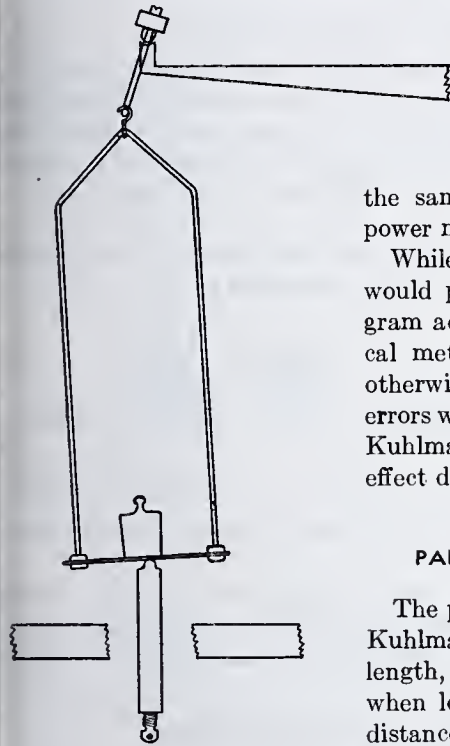


Figure 2. Stirrup

may be increased by the use of a mounted magnifier. Table II gives the results of two representative series on the same balance with a 1.5-power magnifier.

While errors of this magnitude would prevent attaining microgram accuracy by a nonstatistical method if a balance were otherwise perfect, the larger errors which accompany it in the Kuhlmann balance minimize the effect due to interpolation.

PAN ARRESTMENT ERROR

The pan arrestments upon the Kuhlmann balance are of fixed length, while the bows stretch when loaded. As a result, the distance from the stirrup arrestment to the bottom of the pan increases with increasing load.

When the rigid pan arrestment

touches the stretched, freely swinging pan, it tilts the pan and, consequently, the stirrup, as illustrated in Figure 2.

Table II. Accuracy of Observations

No. of Observations	$\sigma, \gamma$	$E_{99}, \gamma$
10	0.6	1.6
10	0.5	1.3

This effect may occasionally be avoided if the center of gravity of the pan and load system falls exactly above the pan arrestment. The farther the load is from the arrestment, the more difficult it will be to secure this adjustment. This leads to difficulties in weighing absorption tubes which are ordinarily placed some distance above the pan arrestment. Even if the center of the pan-load system is above the pan arrestment, the mechanical instability of this system with respect to its point of support makes it impossible to secure uniformly reliable vertical lifting. Any tilting causes an error upon the subsequent releasing of the beam, due to faulty replacement of the flat bearing on the end knife. The magnitude of the error is dependent not only upon the load but upon the method of loading. The error was examined by statistical procedures similar to those set forth in succeeding sections. In one series of observations the pans were arrested and in an otherwise identical series the pan arrestments were removed. This error does not follow a Gaussian probability curve at any load. With 9-gram absorption tubes it appears to have a "standard deviation" in the neighborhood of

1.5 micrograms, corresponding to  $E_{99}$  of 3.9 micrograms. Closer evaluation is of little interest because the error may be eliminated by substituting flexible arrests for the fixed arrests provided by the manufacturer. A simple design for such arrests is shown in Figure 3. For safety, the arrestment should be compressed by loads of less than 1 gram.

The author was not able to find spring wire sufficiently fine to give the delicate touch desired, but found that small lengths of sponge rubber are satisfactory for this application. These are cut so small in diameter that they slide in and out freely. They support a cylindrical pin which moves up and down in the hollow cylinder of the arrestment as impelled by the motions of the pan.

ERROR OF THE CENTER BEARING SYSTEM

Because temperature inequalities strongly affect the zero point of a balance, a Kuhlmann balance was equipped with aluminum shields to prevent body heat from causing temperature changes and with a 20-cm. (8-inch) extension handle for manipulating it from a distance. As changes in the rider could also cause shifts in zero point, the rider was not touched during a long series of observations. Elaborate systems of baffles were also installed in efforts to prevent fluctuations due to possible eddy currents set up by the motion of the beam. All these precautions were insufficient to prevent a considerable residual error on simply arresting and releasing the beam.

By means which are given in detail below, it was possible to establish the fact that this error was due mainly to the operation of the arresting mechanism. Accordingly, the pans and stirrups were removed and the balance was treated as a pendulum, with only its center bearing system in operation. Since the pans of the Kuhlmann balance were found to be unequal in weight, it was necessary to correct the equilibrium adjustment by about 200 mg. This cannot be accomplished with the equilibrium nut, even by moving it through the entire length of the threaded section of the bar which carries it. The support of the threaded bar itself can be rotated about its vertical axis, however, and by thus rotating the bar away from its normal position parallel to the beam, the beam could be balanced roughly with the adjusting nut and more accurately with the rider. A typical series of observations with the balance in this condition is given in Table III. All the precautions mentioned above were observed during this experiment.



Figure 3. Flexible Arrest

In this particular balance, then, the standard deviation of the residual error at the center bearing alone is of the magnitude of 1.2 micrograms, even when loaded with 15.8 grams less than the minimum load under service conditions.

The identification of the residual error of the Kuhlmann balance as an error of the center bearing system can also be achieved by a simpler manipulation of the balance. It is a fairly easy matter to lower the end arrestments so that they just miss the arrestment points on the stirrups. Under these conditions there can be no relocation of the positions of the flat end bearings with

respect to the knives if the pans are not touched. To make sure of this point, it is necessary to lower the pan arrestments so that they do not touch the pans. The residual arrestment error found

Table III. Center Bearing Error

No. of Observations	$a$ , Standard Deviation $\gamma$	$E_{99}$ , $\gamma$	$b$ , Standard Deviation from Reading Error $\gamma$	$\sqrt{a^2 - b^2}$ , Standard Deviation of Residual Error $\gamma$	$E_{99}$ , Residual $\gamma$
10	1.5	3.9	0.9	1.2	3.2



Table IV. Center Bearing Error

No. of Observations	Load <i>Grams</i>	<i>a</i> ,	<i>E</i> <sub>99</sub>	<i>b</i> ,	$\sqrt{a^2 - b^2}$ ,	<i>E</i> <sub>99</sub> Residual
		Standard Deviation		Standard Deviation from Reading Error	Standard Deviation of Residual Error	
		$\gamma$	$\gamma$	$\gamma$	$\gamma$	$\gamma$
10	39.9	0.78	2.0	0.63	0.46	1.2
10	79.9	1.00	2.6	0.63	0.78	2.1

by this method, as described below, must be mainly due to operations at the center bearing (see, however, the last section of this paper).

If the error in the center bearing system were due to a shift in the relative positions of the center of gravity of the beam and the parts of the knife which support the beam, successive releases of the beam would cause errors which should increase in proportion to the increase in load on the center bearing. That this is approximately true is shown from the experiments recorded below.

**EXPERIMENTS.** A factory-reconditioned Kuhlmann balance which had been used for student weighings was the subject of the following observations. The reading error had been reduced by the use of a magnifier in addition to the concave mirror supplied by the manufacturer. The stirrup arrestments were lowered, so that neither touched its corresponding arrestment points, and the pan arrestments were disconnected. It was found that the total load on the center knife when the pans were unloaded was 39.9 grams and when fully loaded, 79.9 grams. The balance was shielded with aluminum and was operated with a long handle. The rider was not moved during the observations. The results are recorded in Table IV.

$0.78 \gamma \times 39.9/79.9 \gamma = 0.39 \gamma$  instead of  $0.46 \gamma$  found. These figures give a false sense of accuracy, since only the first digits after the decimal are significant. It can be concluded that the increase in the residual error is roughly in proportion to the increase in load. Since it is improbable that the error is due to any mechanical defect at the end knives under the conditions of the experiment, it is reasonable to conclude that it is due to a small shift in the point of suspension of the beam with respect to the center of gravity or, in other words, to an error in the center bearing system.

A third method for locating and roughly evaluating this error is given under Total Beam Arrestment Error.

We may thus conclude that in the Kuhlmann balance there is a residual error after correction for the error of interpolation, even when the center bearing is the only one in operation, as well as under conditions which make it improbable that there will be any end-bearing error.

A careful examination of the beam showed that no parts were loose and that the pointer always returned to the same point of the scale on arresting, thus showing that the error was not caused by a loose arrestment. This same error has been observed on numerous occasions with experimental balances having imperfectly ground center knives. It is probably due to progressive microscopic disintegration of the knife bearing, caused by faulty polishing or subsequent chipping. This error was noted by Thiesen in 1886 (37) and was subsequently commented on by Heyl and Cook (11). Schmerwitz (33) has shown that changes in form do take place in the knives and this is also made apparent by the measurements recorded in the last section of this paper. Such progressive disintegration would be capable of causing a load-dependent error of the type found above.

Manley (17) made graphs of the rest point plotted against the number of oscillations of a balance in a study similar to that described here (Reading Error). In these plots he observed both drifts and sinuosities and ascribed both to unequal heating effects. The author agrees, on the basis of experiments described under Error Due to Temperature Gradient, that the drifts were probably due to temperature gradients. He does not agree that sinuosities of the sort observed by Manley are to be attributed to temperature gradients, since experiments in this laboratory with much larger gradients failed to give sinuosities. He is more inclined to attribute them to imperfect bearings, a

source of error which Manley overlooked. It will be noted in Manley's article on the subject that compartmentation stopped the appearance of the sinuosities, an effect ascribed by him to marked reduction in temperature gradients in the vicinity of the beam. Actually, the sinuosities developed in a Bunge balance and the beam was protected in a Gallenkamp balance, so that there is no basis for comparison of the effects with and without compartmentation.

Manley also attributed discontinuities of the sort observed by Thiesen and by Heyl and Cook, referred to above, to shifting of adjusting screws. The author has observed the same effect frequently in balances without any adjusting screws and therefore feels that they are more properly ascribed to defective bearings.

#### TOTAL BEAM ARRESTMENT ERROR

To evaluate the total beam arrestment error of a Kuhlmann balance, the balance was shielded with aluminum shields and operated with a long handle, flexible pan arrests were installed and the rider was not touched during the complete series of observations. The values obtained are given in Table V.

Table V. Beam Arrestment Error

No. of Observations	Load on Pan <i>Grams</i>	<i>a</i> , Standard Deviation	<i>E</i> <sub>99</sub>	<i>b</i> , Standard Deviation from Reading Error	$\sqrt{a^2 - b^2}$ , Standard Deviation of Residual Error	<i>E</i> <sub>99</sub> Residual
		$\gamma$	$\gamma$	$\gamma$	$\gamma$	$\gamma$
100	0	1.9	4.9	0.9	1.7	4.5
100	20	4.3	11.2	0.9	4.2	11.1

These two series are sufficiently large to permit the plotting of probability curves. The deviations from the mean were plotted in each instance. In the first set they were found to correspond well with a Gaussian curve and in the second to deviate somewhat. We may therefore conclude that treating the results of observations on this balance by ordinary statistical analysis is a reasonable procedure.

Careful examination of this balance shows that the manufacturer has supplied it in such adjustment that the left flat stirrup bearing is never separated from the corresponding knife bearing. Only the right stirrup bearing and the center knife are separated from their corresponding bearings when the arrestment mechanism is operated. An inquiry addressed to Dr. Kuhlmann elicited the information that all his microbalances were adjusted in this manner when they left the factory. Accordingly it is to be anticipated that any mechanical defects in the placement of the end bearings on release after arrestment would affect the right bearing only. On this assumption, the magnitude of the errors affecting the center and right bearings may be calculated.

If we represent the center bearing error by  $a$  and the right bearing error by  $b$ , both values taken at zero load on the pans, the increased error on the center bearing due to loading will be  $(79.9/39.9) a$  and the increased error on the end bearing due to loading will be  $(27.9/7.9) b$ . (The weight of a pan and a stirrup is 7.9 grams.) We may then solve the following equations:

$$\begin{aligned} a^2 + b^2 &= 2.80 \text{ (square of the residual error at zero load)} \\ (79.9/39.9)^2 a^2 + (27.9/7.9)^2 b^2 &= 17.68 \text{ (square of residual error at full load)} \end{aligned}$$

Solving,  $a = 1.43$  (center bearing error at zero load on pan)  
 $b = 0.87$  (right bearing error at zero load on pan)

The value thus obtained for the center bearing error is of the order of magnitude found at a different time on the same balance by the method summarized in Table III.

It cannot be argued that the bearing errors found on arrestment and recorded here are peculiar to the particular balance investigated in this laboratory, since the author has had an opportunity to investigate sixteen Kuhlmann balances in various laboratories and has found all to have errors of the same order of magnitude.



The conclusion that the end bearing which is arrested is affected by an error due to the operation of arresting may be vividly demonstrated by raising the arrestments under the left stirrup points until both stirrup bearings arrest when the mechanism is operated. Observations with this adjustment are:

No. of Observations	Load on Pan	Standard Deviation $\gamma$	$E_{99}$ $\gamma$
10	0	11.3	29.5

When the pans are loaded, the magnitude of the error increases still further. With such a large error, the "standard deviation" is not to be regarded as being closely approximated by a few observations. The highly erratic behavior summarized by these figures, however, shows that one of the major factors causing the Kuhlmann balance to perform more accurately than many balances with similar rudimentary arrestments is the omission of the arrestment of one bearing. Two questions come to mind immediately: Why does the arrestment of the third knife produce such a huge increase in error and what, if any, increase in precision may be obtained by omitting the arrestment of the second end knife?

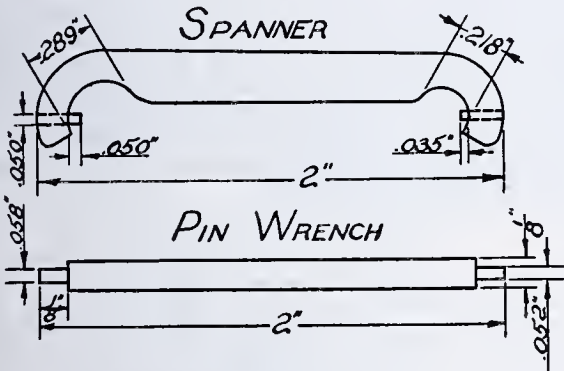


Figure 4. Spanner and Pin Wrench

The motion of the beam may be examined under a microscope at any point desired by mounting a horizontal microscope near the point of interest and a sheet of paper back of that point with sufficient illumination to make the silhouette visible. Such an examination at the moment of arrestment shows the reason for the huge error when both end knives are arrested. With the straight fall-away type of arrestment characteristic of the Kuhlmann balance, it is impossible for the mechanism to arrest all three knives without lifting the end bearings first. If the center bearing were arrested first, the beam would be lifted at the same rate as the arrestment arm and at least one of the stirrups would never be overtaken. Arresting the end bearings first necessarily results in lifting the bearings while the beam is oscillating. This causes violent chattering motion which is plainly visible under the microscope. Hence this elementary type of arrestment is not satisfactory when the manufacturer desires to extend the protection of arrestment to all three bearings.

Curiously enough, the same argument does not apply when only two bearings are arrested. By making the left pan and stirrup combination heavier than the right, the oscillation of the beam may be terminated by catching the beam with the two end arrestments before any knife is lifted. The right knife is then lifted immediately. The weight of the left stirrup in contact with its knife prevents the beam from oscillating further and the whole beam is finally lifted from the stationary center bearing. This gives a tremendous improvement in the arrestment action, but observation with the microscope shows that chatter is not entirely eliminated. As a result, the knife which is arrested, supposedly as a precautionary measure to protect it, suffers incessant pounding during the process. As is demonstrated below, this actually causes it to deteriorate more rapidly than the knife

which is not "protected" by an arrestment. Thus, in this instance, a faultily designed arrestment was worse than no arrestment at all.

An increase in both accuracy and lifetime of the Kuhlmann balance may be secured by readjusting it so that neither end arrestment works and both knives remain permanently in contact with their flat bearings. To accomplish this, two special tools, a spanner and a pin wrench, shown in Figure 4, are required. The other end of each wrench is made to fit similar parts of the pan arrestment. These are located within the glass base.

The readjustment should be carefully performed, so that both arrestments come as close as possible to arresting without actually succeeding. The course of the readjustment may be observed by using a brightly illuminated sheet of white paper as a background for the observation of the contact or separation of each end flat bearing from its knife. The author's experience over a considerable period of years is that this single readjustment of the Kuhlmann balance is the most effective that can be made easily to improve its performance and that it eliminates essentially all of the end-knife arrestment error. This procedure is certainly to be regarded as a compromise due to faulty arrestment design. While it is highly desirable that all three knives of a balance should be arrested, careful experimentation has revealed no method for accomplishing this without sacrifice of accuracy on the Kuhlmann balance short of building in a complete new arresting system.

Another change which will improve the action of the arresting mechanism is the installation of a Conrady pointer brake (5). This is a light wire mounted on knives near the pointer with a small arm to make contact with a moving part of the arrestment. It is adjusted to rub lightly against the pointer until after the knives are released and then to be swung free by the contact of the arrestment part on its arm. While this does not increase the accuracy greatly, it does assist in the protection of the arrested end knife, since its action is to stop the oscillation of the beam before the knife is lifted. The author recommends the use of a Conrady pointer brake with all precision balances to guard against accidental violent fluctuations of the beam.

To aid in comprehending the problem of mechanical design posed by microchemical balances, we may calculate the magnitude of the changes in arm length which are responsible for the observed end knife error on arrestment. Since the length of a single arm of the balance is 35 mm., the deviation in arm length which would be responsible for an error of 0.9 microgram at 7.9-gram load is  $\frac{35 \times 0.9}{7.9 \times 10^6}$  mm. or 4 m $\mu$ . It is not surprising to find a balance with a random deviation in arm length upon arrestment of the order of 0.00000025 cm. (0.0000001 inch), yet it is obvious that this error must be traced and eliminated if the desired accuracy is to be attained.

Table VI. Rider Error					
Experiment	No. of Observations	Standard Deviation $\gamma$	$E_{99}$ $\gamma$	Standard Deviation of Residual Error $\gamma$	$E_{99}$ Residual $\gamma$
(1) Reading only	10	(a) 0.5	1.3	$\sqrt{b^2 - a^2} = 0.6$ (center bearing error)	1.6
(2) (1) + arrest	10	(b) 0.78	2.0		
(3) (2) + rider adjustment	10	(c) 1.6	4.2	$\sqrt{c^2 - b^2} = 1.4$ (rider error)	3.7

RIDER ERROR

The presence in the Kuhlmann balance of an error due to inaccurate placement of the rider in the notches of the beam has been commented on by Ramberg (29) and Schwarz-Bergkampff (34). To eliminate this error a bar rider with channel beam has been introduced by Seederer (35) and Gattoni (9) of the Seederer-Kohlbusch Co. A similar device has also been proposed by Ramberg and introduced abroad by the Sartorius Werke (14). Since most commercial balances are not designed to eliminate the rider error, its magnitude must be evaluated in order that the performance of the balance at all loads may be understood. This may be accomplished by making two series of observations.



the first in which the rider is never moved, the second in which it is reset as accurately as possible after each reading. The usual statistical treatment then permits calculation of the error. A typical experiment of this sort is recorded in Table VI. The experiments were performed on a Kuhlmann balance shielded against body heat and with neither end bearing arresting.

The observations recorded above represent the errors to be expected in weighing a sample on a greatly modified Kuhlmann balance under ideal conditions. Heat errors were eliminated, pan arrestment and end bearing arrestment errors were not present, and great care was taken with each setting of the rider to secure the best adjustment which could be made by eye. This experiment has been repeated on numerous occasions with closely concordant results.

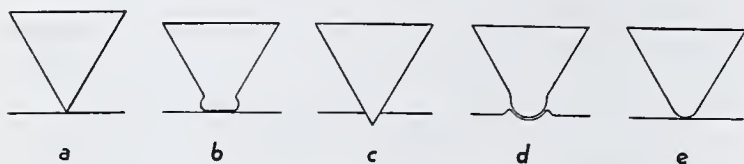


Figure 5. Contact of Knives with Flat Bearings

By a further modification of the balance, even the rider error may be eliminated. The substitution of a 0.5-mg. or 1.0-mg. rider for the 5.0-mg. rider ordinarily supplied with the Kuhlmann balance eliminates the rider error but makes it necessary for the analyst to use milligram weights. If real accuracy is desired, this procedure is recommended. Under these conditions, accuracy of the order of magnitude of that given in the second experiment may be achieved.

Schwarz-Bergkampff (34) examined a Kuhlmann rider bar for differences in the machining of the notches and found none great enough to affect weighings. Manley (23) notes that errors in machining rider bars do occur, however, and recommends that the user provide apparatus to protect himself against such errors. The process of checking the accuracy of machining of a rider bar may be accomplished in at least two different ways. Use of a microscope with an ocular micrometer, as described by Schwarz-Bergkampff, is not thoroughly reliable, since progressive errors might be missed. A better method is to use a microscope mounted on a micrometer screw of sufficient length to cover the entire beam without resetting. A simpler method which is open to the analyst is to construct a 50-mg. rider of platinum with as nearly as possible the dimensions of the Kuhlmann rider. The rider bar may then be calibrated by balancing each new setting of the rider with milligram weights. Only the former method has been used in this laboratory. The Kuhlmann beam was measured with a 100-mm. Gaertner micrometer microscope and 39 notches were found to deviate 1 microgram or more from the average while 8 deviated 2 micrograms or more. None was off by 3 micrograms. The cumulative error was 2 micrograms at the fiftieth notch.

These measurements indicate that even better machining is necessary to secure true microgram accuracy. They are not thoroughly reliable, however, because of the impossibility of deciding by a microscopic examination the exact point at which the rider will seat. Some of the notches had appreciable burr which would have thrown the rider off by more than the amount indicated by the microscope. This leads to the conclusion that rider notches should probably be ground and not milled, although a small burr from milling might be removed by subsequent grinding. The user who wishes to secure the greatest possible accuracy from his instrument should first examine all notches with a hand magnifier and remove any burr with a sharpened match stick, since this is too soft to affect the machining of the notch. He should then calibrate the notches with a platinum rider. Alternatively, the use of a lighter rider, as recommended above, eliminates the error on this particular type of balance.

### Curvature of the Knives

The replacement of a flat bearing on a perfect knife should always yield the same arm length, even when the replacement is not performed with precision. One might inquire, then, why arrestment errors are possible. They would not be if actual balances were the same as the "geometrical balances" with which balance theory usually deals. For the purposes of the present

argument, however, it is necessary to set aside the usual assumption that the knife bearings form perfect geometrical straight lines.

Figure 5 shows several possibilities of the contact of knives with flat bearings. In *a* we have the picture as it is usually represented, a perfect straight line in contact with a perfect plane. In practice this is impossible of attainment because of mechanical deformation. If we imagine a soft knife in contact with a hard flat, we shall have the type of contact shown in *b*. This case is frequently observed and gives rise to rapid dulling of the knife edge. A hard knife in contact with a soft flat gives the figure shown in *c*. This case is also easily demonstrated in practice and results in permanent injury to the flat bearing. Balances equipped with bearings of types *b* or *c* quickly show erratic behavior. An ideal combination is one in which both knife and flat bearing are constructed of the same material or materials of equal hardness and in which neither is loaded beyond its elastic limit. The picture of the deformation to be expected in such a case is shown roughly in *d* and is the case normally to be expected. If we permit the bearing shown in *d* to roll slightly, the depth of penetration will remain constant and the net effect will be that the circumference of the convex cylinder will move along the plane parallel to the original plane of the bearing but displaced from it by the distance of the depth of penetration. For this reason the figure may be simplified for the purpose of the analysis of the statics (but not necessarily the dynamics) of the balance system as in *e* and we shall treat the knives as if they were cylinders reposing upon planes. This assumption introduces into the geometrical instrument a deviation from the action of a perfect pendulum. The effect was first alluded to by Richarz and Krigar-Menzel (31) and first measured by Guglielmo (10). A more extended study was later made by Schmerwitz (33). Both the calculations and the instruments used by these experimenters are unnecessarily complicated. A simplified treatment of the determination of the radius of curvature of the loaded bearing is given herewith.

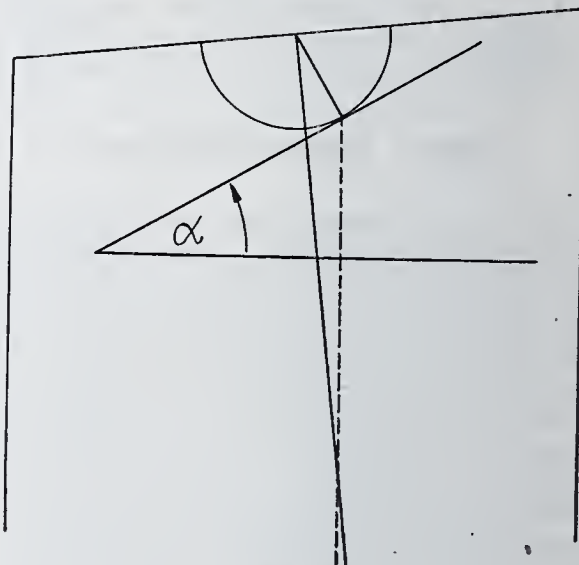


Figure 6

Tilting the case of a sensitive balance will cause the beam to incline from the horizontal until the center of gravity assumes a position below the point of contact of the bearing with the plane. This position is represented in Figure 6. Adding a restoring weight,  $w_r$ , will bring the beam back to the horizontal position as in Figure 7. Moments about the point of contact may now be equated. The mass of the beam is acting through the lever arm  $(r \sin \alpha)$ . The restoring weight is acting through the lever arm  $(L - r \sin \alpha)$ . Because  $(r \sin \alpha)$  is so small with respect to  $L$ , it may be neglected in the second expression. We thus have:

$$\begin{aligned} Mr \sin \alpha &= w_r L \\ \text{or} \quad r &= w_r L / M \sin \alpha \end{aligned}$$

**PROCEDURE.** In the laboratory, this method of tilting the balance case to determine the radius of curvature of the center knife is much simpler than the procedures described by Guglielmo and Schmerwitz. The tilting is accomplished by the use of gage blocks. If the balance is supported by three legs, two in front and one in back, it may be tilted sideways by the use of two gage blocks, one exactly twice as thick as the other. The thicker block is placed under one front leg, the thinner under the central stud in the rear of the case. If the balance is supported by four



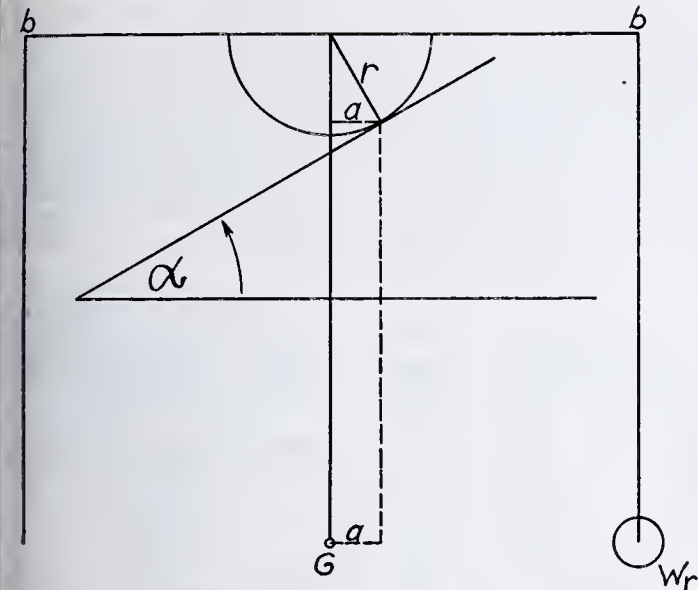


Figure 7

gs, two blocks of exactly equal thickness may be placed under the two legs on one side. The distance between the front legs is measured as accurately as possible, using corresponding parts of the legs as points of comparison. The thickness of the gage blocks is determined with a micrometer. The balance is set for the test on a glass or steel plate or upon flat metal blocks, so that the legs will not sink in when the case is tilted. Under these conditions the thickness of the largest gage block is divided by the length of the case between legs  $\sin \alpha$ . With a microbalance an angle of approximately  $1^\circ$  is usually satisfactory. With analytical balances, angles up to  $3^\circ$  may be used. If gage blocks for  $1^\circ$  are available, the angle may be doubled by taking the starting point with the large block under one leg and the tilted point with the block under the opposite leg. When the case is tilted, the pointer scale is moved with respect to the gravity line. The pointer should, therefore, move through angle  $\alpha$  on the pointer scale if the bearing were perfect. The difference between the angle through which the pointer should move and that through which it actually does move, translated into terms of weight, represents the restoring weight. Since the critical quantity desired is usually a small difference between two larger quantities, each reading must be made with the greatest precision of which the balance is capable. To calculate the restoring weight, it is necessary to evaluate  $\alpha$  in terms of pointer scale divisions. There are two methods for doing this. One is to measure the pointer scale and the distance from the knife edge and set their quotient equal to the sine of the total angle subtended by the scale. From this the fraction represented by angle  $\alpha$  can be calculated. The other method is to lower the center of gravity of the beam a great deal, perhaps by attaching a light clamp to the pointer part of the way down, and then to tilt the case through angle  $\alpha$  and see experimentally how far the pointer moves. With the center of gravity very low, the balance performs as a perfect pendulum with respect to the angle of tilt and the theoretical value can be read off directly from the scale. The length of the beam can be measured conveniently and by dividing by 2 the arm length,  $L$ , is obtained. The mass of the beam is obtained by weighing it on a rough balance. If the balance has been prepared for the experiment by the removal of the pans and stirrups, only the mass of the beam is involved in the expression. When performing the determination in this manner, it is sometimes necessary to make a considerable readjustment of the zero point with the balancing nuts to bring the pointer to the scale. The determination may also be performed with pans and stirrups in place, but even in this case  $M$  is the mass of the beam only, provided that the sensitivity remains constant with load. That the radii of the end knives will not affect the result can be seen from Figure 7 if we imagine the point "end bearings" shown  $(b - b)$  to be replaced by cylinders with planes tangent at the same points as those in the illustration. This change will not affect the arm length. With the quantities found thus the radius of curvature can be calculated. An example of an actual determination is given herewith.

Measurement of Center Knife Radius of Kuhlmann Balance.  
Calculation of  $\alpha$  in Pointer Scale Units.  
Thickness of gage block, 6.35 mm.  
Length of case, 339 mm.

$$\sin \alpha = \frac{6.35}{339} = 0.01873$$

Length of Kuhlmann pointer, 139 mm.

If the bearing were perfect, tilting the case through  $\alpha$  should displace the pointer scale from the perpendicular position of the pointer by  $139 \sin \alpha$  mm. or 2.604 mm. But 100 Kuhlmann scale units = 1.938 mm.

$$\text{Hence } \alpha = \frac{(2.604)(100)}{1.938} = 134.35 \text{ scale units}$$

Thus tilting the case through  $\alpha$  should shift the scale by 134.4 units of rest point if the bearing were perfect. If we use deflection instead of rest point, tilting the case through  $\alpha$  should cause a displacement in the deflection of the pointer of 269 scale units.

Calculation of the Radius of Curvature. The displacement observed on tilting through  $\alpha$  was 205 scale units. This means that tilting the case caused a shift of the pointer from the perpendicular position of  $(269 - 205)$  or 64 scale units. The sensitivity of the balance was, 1 unit of deflection =  $\frac{0.1}{108}$  mg. per scale

$$\text{unit} = \frac{1}{1080} \text{ mg. per scale unit. Hence } 64 \text{ units} = \frac{64}{1080} \text{ mg.} = 0.059 \text{ mg.}$$

Weight of beam, 24,029 mg.

Length of arm,  $L$ , 35 mm.

Hence,

$$r = \frac{(0.059)(35)}{(24,029)(0.01873)} = 0.00456 \text{ mm. or } 4.6 \mu$$

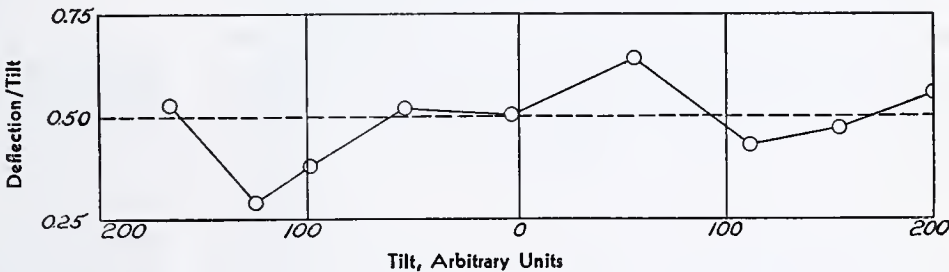


Figure 8. Curvature of Knife Edge  
----  $r = 11.2 \mu$

The equation for the calculation of the radius of curvature is the most important of all the expressions at present applicable to a precision balance because it enables the user to evaluate the constant which is most directly responsible for reproducibility in the instrument as contrasted to sensitivity. It is conceivable that a method may be found for preparing knives which are tiny perfect cylinders. However, at the present time, such a technique has not been described. Any larger radius of curvature than the minimum obtainable with the material used is to be regarded as a flaw and is always accompanied in practice by more or less erratic behavior on the part of the instrument. A subsequent communication will contain details upon the application of this test method to the preparation of precision knife-edges. As a result of these experiments, we regard  $0.25 \mu$  as being the maximum radius permissible in a precision instrument. It is to be emphasized, however, that this figure represents the maximum permissible when the objective of the manufacturer has been to produce a mathematical line for the bearing. If he were to find a process for preparing a perfect cylinder of slightly larger radius, this might actually be preferable.

If we consider that the center flat bearing of the balance is perfectly plane and the knife is ground to a mathematical straight line, the contact will be cylindrical, as shown in Figure 5,  $d$ , because of the mechanical deformation of the bearings under the load impressed upon them. In practice, this is the nearest approach to the ideal case. Actually, it is frequently found that the flat bearing deviates considerably from perfect flatness and the knives are not perfectly ground. Even in this case the equa-



tion given above provides a means for learning what the condition of the bearing is. An examination of Figure 6 will show that the angle of tilt of the beam produced by tilting the case through a given angle will be increased by raising the center of gravity. By raising it sufficiently, it is possible to make the balance so sensitive to tilt that the excursion of the pointer may be subdivided into a considerable number of angular increments. If the case be tilted through successive angular increments by means of a micrometer screw under one end, a curve may be constructed by plotting the deflection per unit tilt of the case against the number of micrometer units that the case is tilted. The value of the plot at zero is indeterminate. With a perfect cylinder this plot should be a horizontal straight line. If there are irregularities in the edge, the apparent radius of curvature will differ with different angular increments and the plot will not be a horizontal straight line. From the shape of the curve it is possible to reconstruct roughly the shape of the knife-edge. An example of the appearance of such a curve is given in Figure 8. The "radius of curvature" at any angle may be obtained by multiplying the ordinate at that angle by 22.4.

This procedure is suggested by the work of Guglielmo (10) and is much simpler than that of Schmerwitz (33). With either this procedure or that of Schmerwitz, the author has found it possible to locate deviations from the form of a perfect cylinder only with relatively poor knives.

It will be obvious immediately that an imperfectly ground knife-edge in the center bearing will cause the instrument to behave erratically. If the distance from the center of gravity to the point of suspension varies irregularly with the angle, the zero point of an oscillating balance will be irregularly dependent upon the amplitude of the oscillations. This is a possible explanation of the "sinuosities" of the curve of the zero point of a damping balance found by Manley (17). If sharp edges protrude from the knife, the wear will be uneven at different parts of the knife and the plot mentioned in the preceding paragraph will be unstable with time. This possibility was discussed under Error of the Center Bearing System.

Because of the curvature of the knives, it is possible that the center of gravity of the beam may be raised to such an extent that it is actually above the point of contact. As long as it remains below the turning point, the instrument will be stable. When the center of gravity is just at the knife-edge, the beam will tilt through the same angle as the case and the pointer will appear to remain fixed with tilt. These cases were pointed out by Conrady (5). While Conrady appeared to look with some favor upon the possibility of the preparation of precision balances with such adjustments, the author's experience has been that such an adjustment cannot be obtained in practice except with a very poorly ground knife and that a knife which is so poor as to permit adjustment to Conrady's "autostatic" state is so poor that it will not give reproducible weighings.

### Radii of the End Knives

If the center knife were a perfect cylinder, its radius could be quite large without affecting the reproducibility of weighings. With the end knives the situation is different. Here each increase in radius of curvature carries with it the necessity for a corresponding increase in the precision of replacement of the flat bearings by the arresting mechanism, if precise weighings are to be obtained. Hence the problem of the tolerances to be set upon arrestment design is inextricably bound up with the radii of the end knives.

A manufacturer may determine the radii of the end knives by inserting them in a special balance as center knives before mounting them. This procedure is hardly available to the user of an instrument, who will be forced to resort to such a method as that outlined below.

**PROCEDURE.** The compensating link of the stirrup is temporarily replaced by a special link which immobilizes the wrist action in the direction of the beam but permits the pan to swing freely in the direction perpendicular to the beam. This is illustrated in Figure 9. Hanging a weight on one side or the other of this special link will tilt the stirrup and cause it to come into

contact with a different part of the knife-edge, just as tilting the case alters the point of contact of the center knife. The angle of tilt of the stirrup can be determined by attaching to it a long, light pointer made of fine, stiff wire. An engineer's scale graduated in  $\frac{1}{64}$  inch is mounted in the case with stiff wax and the wire pointer is bent to sweep immediately above the scale. It is convenient to use a magnifier in reading the scale. A change in zero point caused by tilting the stirrup with a given load attached corresponds to the restoring weight of the equation and the radii of the end knives may be determined with its use. An example of such a measurement is given below:

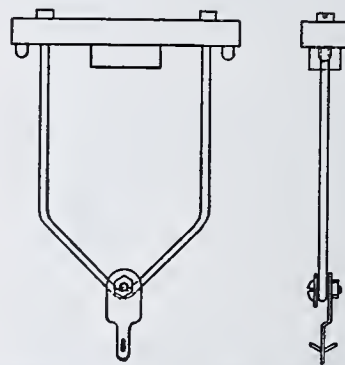


Figure 9. Radii of End Knives  
Left, front view. Right, bent brass pin soldered in to hold pan and weights

*Measurement of Right-End Knife Radius on Kuhlmann Balance. Length of auxilary pointer, 5.25 inches*

Pointershift on tilting stirrup

$$\frac{26.5}{64} \text{ inch,}$$

$$\sin \alpha = \frac{26.5}{(64)(5.25)} = 0.078$$

The special stirrup with its load was adjusted to 10 grams. Shifting this weight through angle  $\alpha$  caused a displacement of 0.288 mg. Hence  $w_r = 0.288 \text{ mg.}$

$$r = \frac{(0.288)(35)}{(10,000)(0.0789)} = 0.01278 \text{ mm.} = 12.8 \mu$$

The fact that the end bearings are also not mathematically straight lines gives rise to the stirrup arrestment error noted in balances (31). Unless the mechanism is designed to secure the return of the end bearings to exactly the point from which they were taken on arrestment, the arm length will vary with each arrestment and weighings will not be reproducible. If the apparent radius of curvature varies with the angle, the end knives will also contribute to an irregular error dependent upon the amplitude of oscillation of the balance. This effect would be even more pronounced if the center of curvature varied with the angle. It is true that an imperfect bearing might give rise to a very small error, provided that the imperfection were exactly proportional to the angular displacement from the perpendicular. If, however, any point on the bearing surface had its center of curvature displaced horizontally from the average center of curvature by so much as 13 Å, it would cause an error of 1 microgram at full load on the Kuhlmann. This follows from the fact that at full load the end knife of the Kuhlmann has a load of 27,000,000 micrograms. To cause an error of 1 microgram would require a change in arm length of  $\frac{35 \text{ mm.}}{27,000,000}$  or 13 Å.

When the Kuhlmann balance was first purchased, the author's examination showed that all three knives had radii between 3.0 and 3.5  $\mu$ . Six years later, the center knife had a radius of 4.5  $\mu$  and the left knife 5.2  $\mu$ , while the right knife showed a radius of 12.8  $\mu$ . From these results it will be observed that the left knife, which was not arrested, maintained its radius of curvature better than the right knife which was arrested. This emphasizes again the faulty nature of the Kuhlmann arresting mechanism.

In the examination of balances other than the Kuhlmann, certain of the errors enumerated above and still other errors not present in the Kuhlmann will be found. In view of the number of factors which can cause microgram errors, it is impossible to predict the probable accuracy of any balance without a thorough trial under the intended conditions of use. Since different instruments vary in their susceptibility to environmental influences and different laboratories vary widely in the suitability of their balance rooms for susceptible balances, various instruments will show wide divergences of performance in different laboratories.



The prospective purchaser of an instrument should calculate for himself the maximum error which he can tolerate in weighing and should then purchase an instrument only on condition that it can meet this performance specification under the conditions prevailing in his laboratory. Specifications of this sort rigidly adhered to will speed the day when manufacturers will cease to confuse sensitivity with accuracy.

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## Colorimetric Determination of Tin with Silicomolybdate

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A rapid and accurate colorimetric determination of tin for concentrations ranging from 0.0005 to 0.5% is described. The method is an excellent routine procedure requiring no special technique or apparatus other than a comparator or filter photometer. Tin is separated by distillation and determined colorimetrically in the distillate. Five determinations can be run in 2 hours. Effects of variables such as time, acidity, and concentrations of reactants and of interfering ions have been investigated.

THE determination of tin, particularly in small concentrations, is of vital importance in analyses of steels and non-ferrous alloys. Since much of our present steel is being prepared from scrap which may contain tin, and since the presence of tin even in small concentrations is likely to be injurious, added stress is placed on the determination of tin in steel and scrap. Generally, small amounts of tin, ranging from 0.001 to 0.05%, will be found in most steels with the exception of tinplate or special melts. The determination of small quantities of tin in food, biologicals, fumes, and organic matter is also important.

Since small quantities of tin are present in the material to be analyzed, gravimetric or volumetric procedures (10, 14) are unsatisfactory.

Spectrographic analysis (9, 11) offers an excellent solution to the problem. However, because of the skill and expense required, spectrographic methods are beyond the reach of the average laboratory.

An examination of colorimetric procedures suitable for the determination of tin in the concentration range of 0.0005 to 0.5%

was undertaken. The use of organic reagents (16) for the colorimetric determination of metallic ions has increased considerably. Newell, Ficklen, and Maxfield (8) made a critical study of the use of cacotheline test and found that it cannot be regarded as specific. Where interfering substances are absent, the test may be used for detection or confirmation of the presence of small amounts of tin. Experiments with cacotheline in the authors' laboratory failed to give satisfactory quantitative results. Tartakovskii (12) used hematoxylin for the colorimetric determination of tin. The concentration of tin required ranges from 0.3 to 2 mg. per liter of solution. The sensitivity is, therefore, too great to obtain accuracy in the ranges of tin concentration in which the authors are interested.

Hanssen (2) recommended comparison of colloidal tin sulfides. The method is unsatisfactory, since traces of elements and the presence of sulfur caused by the bromine used in the oxidation of stannous ion result in large deviations.

A number of other procedures have been suggested. Clark (1) used 4-methyl-1,2-dimercaptobenzene to determine 2.5 to 30 parts of tin per million parts of solution. Diazin Green S (K) (16) has been used to detect as little as 0.02 mg. of tin per drop of solution. Lowenthal (6) used a mixture of ferric chloride and potassium ferricyanide for the estimation of stannous ions.

Longstaff (5) used the reaction based on the reduction of ammonium molybdate by chlorostannous acid to detect tin to the extent of 1 part in 1,500,000. Hüttig (3) developed conditions under which he claimed quantitative results: separation of the sulfides of arsenic, antimony, and tin, dissolving in 1 to 1 hydrochloric acid, boiling to remove hydrogen sulfide, and reduction with zinc. The reduced tin solution is filtered into 100 ml. of an ammonium molybdate solution and after 0.5 hour is compared with a similar solution prepared from a standard tin solution. Attempts to duplicate these results in this laboratory failed.



## EXPERIMENTAL WORK

In order to determine the cause of the erroneous results obtained using the method of Hüttig (3), an investigation similar to that of Truog and Meyer (13) and Woods and Mellon (15) on the molybdenum blue reaction was conducted. This included the effect of acidity, reagents, method of reduction, and time of reaction upon the color developed. The experiments were performed by adding a standard chlorostannous acid solution to an ammonium molybdate reagent solution.

Variations in the acidity of the solution under test caused divergent results at high acidities; the blue color of the reduced molybdate complex did not form. At lower acidities inconsistent results were obtained in the intensity and fading rates of the blue color. No conformity to Beer's law was found.

Table 1. Colorimetric Determination of Tin

Tin Taken Mg.	Tin Found		Tin Taken Mg.	Tin Found	
	Photometer <sup>a</sup> Mg.	Comparator <sup>b</sup> Mg.		Photometer <sup>a</sup> Mg.	Comparator <sup>b</sup> Mg.
0.02	0.0196	0.022	0.50	0.500 <sup>c</sup>	0.500 <sup>c</sup>
0.04	0.0392	0.0415	0.60	0.573	0.562
0.08	0.088	0.085	0.70	0.670	0.685
0.10	0.096	0.090	0.80	0.787	0.766
0.12	0.140	0.131	0.90	0.851	0.862
0.16	0.171	0.176	1.0	0.952	0.948
0.18	0.184	0.187	1.1	0.965	0.960
0.20	0.241	0.231	1.2	1.06	1.07
0.30	0.284	0.290	1.5	1.14	1.11
0.40	0.364	0.378			

(Values through 0.18 mg. of Sn determined by micromethod)

<sup>a</sup> 0.5 mg. of Sn on A Scale Fisher AC electrophotometer using 650A filter = 22.5 mg.

<sup>b</sup> 0.5 mg. of Sn at 20 mm. on Duboscq comparator.

<sup>c</sup> Standards.

The ammonium molybdate reagent used by Hüttig (3) is prepared by dissolving 0.3 gram of c.p. ammonium molybdate in 4 ml. of water, adding 2.4 ml. of a 14 *N* sulfuric acid solution, stirring, and adding 3 ml. of a 2 *N* sodium hydroxide solution and 1 liter of water.

Preparation of this solution resulted in formation of a yellow color. An examination of the reagents and their impurities led to the conclusion that the yellow color was due to the formation of a heteropoly compound from the acid ammonium molybdate and the silica impurity in the sodium hydroxide. This was further verified by substituting an equivalent amount of silica-free potassium hydroxide for sodium hydroxide in the molybdate reagent in one experiment and in a second experiment omitting sodium hydroxide and adjusting the acidity by reducing the sulfuric acid content of the reagent. In every case where the silica was removed the yellow coloration did not appear and a very weak, unstable blue color was formed upon reduction of the molybdate reagent with the standard chlorostannous acid solution. Addition of a solution of sodium silicate to the silica-free reagents formed the yellow color quantitatively reducible with chlorostannous acid to the molybdenum blue complex.

A theoretical consideration of the molybdenum blue complexes (4, 7, 17) reveals that reduction of molybdates yields unstable blue reduction complexes, the color being destroyed on changing the acidity. Silicates react with molybdates under suitable conditions to form a yellow color due to the formation of a complex of the composition  $H_4(Si(Mo_6O_{10})_4)_nH_2O$ . This complex is more sensitive to the action of reducing agents and more stable than the reduction complex from the molybdates.

The proper adjustment of the ammonium molybdate-acid-silica ratios was investigated. Too great an excess of silicate formed a deep yellow color which interfered with the colorimetric determination of tin.

Difficulty was experienced in duplicating the intensity of blue color when identical concentrations of tin were determined, owing to the rapid rate of oxidation of the reduced tin. After a number of methods were tried, including the use of inert carbon dioxide atmosphere, it was found that accurate, duplicable re-

sults could be obtained by pouring the silicomolybdate reagent directly into the solution containing the tin after the reduction period, while still in contact with the zinc, and decanting immediately. This procedure excludes the use of zinc dust. Better results were obtained by the use of zinc shot.

The next step in the operation is the separation of tin from various materials preparatory to the colorimetric determination. A distillation procedure proved most rapid and efficient. In this procedure of dissolution most of the arsenic will be removed. The only contaminating substance that may distill over is antimony. Nitric acid must not be present, since it will interfere in the colorimetric determination of the distillate. Numerous experiments were conducted to determine the exact conditions of temperature and distillation rate and the concentrations of reagents to effect the complete, uncontaminated separation of tin.

## REAGENTS

**CHLOROSTANNOUS ACID SOLUTION**, 0.05 mg. of tin per ml. Dissolve 0.1 gram of pure tin in 100 ml. of 1 to 1 hydrochloric acid and dilute to 2 liters with 1 to 1 hydrochloric acid.

**SILICATE SOLUTION**, 1 mg. of silica per ml. Fuse 1 gram pure silica with 5 grams of c.p. sodium carbonate, dissolve in distilled water, and dilute to 1 liter.

**MOLYBDATE SOLUTION**. Dissolve 5.3 grams of c.p. ammonium molybdate in 100 ml. of distilled water, add 10 ml. of concentrated sulfuric acid (specific gravity 1.84), and dilute to 200 ml.

**SILICOMOLYBDATE REAGENT**. Dilute 10 ml. of the molybdate solution to approximately 800 ml., add 2.5 ml. of the silicate solution, and dilute to 1 liter. Mix thoroughly and allow to stand 0.5 hour before use. Prepare a fresh supply daily from the stock solution of molybdate and silicate.

**ZINC SHOT**. Reagent grade, low in arsenic, lead, and iron weighing 0.4 to 0.5 gram per shot.

## PROCEDURE

In case of ferrous metals containing less than 0.05% tin, weigh accurately a 10-gram sample for analysis; for metals higher in tin, use a proportionally smaller sample. Introduce the sample into a 250-ml. Claisen flask and add 200 ml. of a solution containing 15 ml. of concentrated sulfuric acid (specific gravity 1.84) and 30 ml. of concentrated hydrochloric acid (specific gravity 1.19). Add glass beads to minimize bumping. Evaporate to the first fumes of sulfuric acid, or until the residue is semisolid, but not to dryness. In case of nonferrous metals containing more than 0.05% tin, dissolve 1 gram of sample in a 400-ml. tall-form beaker with 15 ml. of concentrated sulfuric acid, 10 ml. of concentrated nitric acid (specific gravity 1.42), and 25 ml. of water. Fume strongly to remove excess nitric acid, transfer to a 250-ml. Claisen flask, and wash the beaker with 1 to 1 hydrochloric acid. Add glass beads and dehydrate.

After dissolution and dehydration of the sample, add 10 ml. of concentrated sulfuric acid. Place a 200° C. thermometer in the main neck of the Claisen flask with the thermometer bulb close to the bottom of the flask. In the other neck place a 50-ml. separatory funnel. Attach the side arm to a water condenser. Receive the distillate in a 100-ml. Erlenmeyer flask. A trap and caustic solution may be connected to the Erlenmeyer flask to prevent the escape of uncondensed hydrochloric acid vapors. Pour 10 ml. of concentrated hydrochloric acid and 15 ml. of 40% potassium bromide into the Claisen flask through the separatory funnel to prevent loss of tin. Transfer 40 ml. of concentrated hydrochloric acid to the separatory funnel. Distill the solution until the temperature of the solution in the flask reaches 138° to 143° C. Add the 40 ml. of concentrated hydrochloric acid drop by drop from the separatory funnel, keeping the temperature between 138° and 143° C. After all the acid has been added, continue distillation until the temperature rises to 150° C. Discontinue heating, disconnect the condenser, and wash it with 1 to 1 hydrochloric acid, collecting the washings in the flask containing the distillate.

The distillate now contains the tin, and requires only evaporation to a definite volume before being ready for the colorimetric determination.

The volume to which the distillate is evaporated before testing depends on the milligrams of tin present. Two procedures have been developed, depending on the concentration of tin.



**MICROPROCEDURE** (tin range 0.02 to 0.2 mg.). Evaporate the distillate to less than 6 ml., dilute to 6 ml. with 1 to 1 hydrochloric acid, and transfer to a 50-ml. Erlenmeyer flask. Heat to boiling, add 2 zinc shots, and shake while boiling for 1 minute. Pour 20 ml. of the silicomolybdate reagent into the reaction flask, mix, and decant immediately into a glass-stoppered Erlenmeyer flask, to separate the solution from the zinc within 10 seconds. Simultaneously run a standard containing 0.1 mg. of tin per 6 ml. by the same procedure. Compare the resulting colors after standing 5 minutes.

**MACROPROCEDURE** (tin range 0.2 to 1.0 mg.). Evaporate the distillate to about 25 ml., dilute to 30 ml. with 1 to 1 hydrochloric acid, and transfer to a 250-ml. tall-form Erlenmeyer flask. Heat to boiling, add 10 zinc shots (approximately 4 to 5 grams), and shake while boiling for 1 minute. Pour 100 ml. of the silicomolybdate reagent into the reaction flask, mix, and decant immediately into a glass-stoppered Erlenmeyer flask to separate the solution from the zinc within 10 seconds. Simultaneously run a standard containing 0.5 mg. of tin per 30 ml. solution by the same procedure. Compare the resulting colors after standing 5 minutes.

No color is found in a solution containing no tin when the 1-second maximum mixing and decanting time is not exceeded. This period of time is more than ample to permit efficient operation.

#### RANGE OF CONCENTRATION AND CONFORMITY TO BEER'S LAW

A number of determinations were run on tin standards containing 0.02 to 1.5 mg. of tin. Solutions containing 0.18 mg. of tin or less were determined by the micromethod and above 0.18 mg. of tin by the macromethod. Each result was checked on both the photoelectric filter photometer (Fisher) and a Duboscq comparator, using 0.5 mg. of tin per 30 ml. as the standard. It is evident from Table I that excellent results, conforming to Beer's law, can be obtained for solutions containing up to a maximum of 1 mg. of tin. Concentrations of tin greater than 1 mg. per 30 ml. of 1 to 1 hydrochloric acid solution gave weaker color intensities than is required by the Beer's law straight-line relationship. In the micromethod a maximum error of 15% was found in the range from 0.02 to 0.2 mg. of tin, which represents an error of only 0.03 mg. of tin when 0.2 mg. is present. The microprocedure as outlined will determine accurately within 1% of the actual value from 0.2 to 1 mg. of tin per 30 ml. of solution.

In all the determinations by both methods, the per cent of tin calculated from a reading with the Duboscq comparator closely checked the results obtained using the Fisher photoelectric filter photometer.

#### STABILITY OF REAGENT AND MOLYBDENUM BLUE COLOR

Experiments on the effect of aging of the silicomolybdate reagent disclosed that the freshly prepared reagent did not give satisfactory results if used immediately. After 24 hours, good results were again obtained because of the decomposition of the heteropoly complex. Therefore, solutions were prepared each morning, or prior to use, allowed to react for 0.5 hour, and any excess was discarded at the end of the day.

The stabilities of the molybdenum blue complexes formed by reducing the silicomolybdate reagent with amounts of tin varying from 0.1 to 1 mg. per 30 ml. of 1 to 1 hydrochloric acid solution were determined by measuring the color intensities on the photometer at various intervals of time. Maximum color intensities are formed in each case within 5 minutes after reduction. For concentrations of tin equal to 0.6 mg. or less, only slight fading occurred in 1 hour. In greater quantities, fading was appreciable in 30 minutes. Readings taken after 5 minutes gave satisfactory results in all cases.

#### EFFECT OF ACIDITY

The acid concentration of the silicomolybdate reagent was varied so as to obtain a final normality of the mixture of reagent and tin solution containing 0.5 mg. of tin ranging from 0.2 to 1.72. Photometer readings at the end of 5 and 60

minutes showed that this wide range of normalities had slight effect on the development or stability of the blue color.

#### EFFECT OF DIVERSE IONS

A study was made of the effect of diverse ions that may be found in the distillate after separation of tin, as described in the procedure upon the blue color. Arsenic has little effect on the color until 5 mg. are present in the 30-ml. aliquot. Antimony interference is slight up to 3 mg. in the 30-ml. aliquot. These concentrations are very high and, therefore, no interference is to be expected in the majority of cases in which small quantities of tin will be determined. Concentrations of iron over 0.2 mg. per 30 ml. depress the color and give a greenish cast to the solution. The method of separation of tin used in the procedure excludes all but traces of these impurities and, therefore, little interference from them is found in an actual determination. In the absence of tin, the presence of antimony may lead to the determination of 0.1 mg. of tin per 30 ml.

#### RESULTS

Results of determinations on standard samples prepared by adding known quantities of tin to 10 grams of tin-free steel showed a maximum error of 4%. Table II shows the results of 45 determinations of National Bureau of Standards and Norfolk Navy Yard steel samples. The maximum deviation is 0.0018% and the maximum per cent deviation 0.0012. Also listed are 26 tin determinations of nonferrous metals. The maximum deviation is 0.023% tin and the maximum per cent deviation is 0.015 in manganese bronzes containing 0.2 to 0.5% tin.

Table II. Determination of Tin

Sample	No. of Detns.	Tin Found %	Deviation %
N.B.S. 25 Si steel <sup>a</sup>	4	0.0063	±0.0005
Steel from Norfolk Navy Yard	6	0.0065	±0.0012
N.B.S. 106, Cr-Mo-Al steel	4	0.014	±0.0005
N.B.S. 73 stainless steel	4	0.0029	±0.0002
N.B.S. 55 ingot steel	7	0.0029	±0.0002
N.B.S. 9C-Bessemer steel	5	0.0015	±0.0001
N.B.S. 33-3% Ni steel	4	0.0039	±0.0002
N.B.S. 72B, Cr-Mo steel	5	0.0073	±0.0009
N.B.S. 126-36% Ni steel	6	0.0168	±0.0008
Mn bronze 133040	9	0.202	±0.005
Mn bronze 133041 <sup>c</sup>	5	0.223	±0.015
Mn bronze 133042	6	0.390	±0.013
Mn bronze 133143 <sup>d</sup>	6	0.415	±0.008

<sup>a</sup> Bureau of Standards analysis of tin = 0.007% includes any As or Sb.

<sup>b</sup> Determinations by micromethod.

<sup>c</sup> Gravimetric determination = 0.20% tin.

<sup>d</sup> Gravimetric determination = 0.43% tin.

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THE views presented in this article are those of the writers and are not to be construed as the official views of the Navy Department.



# Hallett Appointed Associate Editor

## Chapman, Churchill, and Oser Elected to Advisory Board

It is with a deep feeling of satisfaction and personal pleasure that I announce the appointment of L. T. Hallett as associate editor of the ANALYTICAL EDITION of INDUSTRIAL AND ENGINEERING CHEMISTRY, and report the election by the Council of R. P. Chapman, J. Raynor Churchill, and Bernard L. Oser to the Advisory Board.

Dr. Hallett brings to the publication a wealth of diversified experience in the analytical field. He will devote a considerable portion of his efforts to initiating new editorial programs.

The Advisory Board of the ANALYTICAL EDITION has been functioning very actively now for slightly more than a year. The members of the board and your editor have felt that several highly important specialized divisions of analytical chemistry

were not satisfactorily represented by the existing make-up of the membership of the board. Accordingly permission was requested to increase the membership from six to nine. At the most recent meeting of the board, held in Washington, January 22, 1944, the question of what fields should be more directly represented at this time was analyzed and nominations by members of the board were carefully considered. The names of Messrs. Chapman, Churchill, and Oser were presented by the editor to the Council at its meeting in Cleveland, April 5, 1944. I am happy to state that these individuals were unanimously elected.

*Walter J. Murphy*

Lawrence T. Hallett brings to his position as associate editor of the ANALYTICAL EDITION a background in analytical chemistry dating from his associating while an undergraduate at the University of British Columbia with E. H. Archibald, professor of chemistry, who had been a student of T. W. Richards. This early interest in precise analytical methods was deepened while working on a master's thesis on preparation of pure rubidium and cesium chloroplatinates and determination of their solubilities. In fulfillment of his mother's wish that some part of his education be obtained in the United States, he undertook further graduate study in analytical chemistry at the University of Wisconsin.

After a few months of instructing, a full-time graduate fellowship was taken in the development of microanalytical methods for the analysis of lake water residues. After receiving a Ph.D. from the University of Wisconsin in 1928, he had a brief excursion into teaching analytical chemistry at Oregon State College and research on thermal insulation.

In 1933 he joined the Eastman Kodak Company to develop a laboratory for microanalysis and thereby found his long cherished opportunity to prove that micromethods were both accurate and rapid and entirely suited to use in industry. After establishing the laboratory, he became interested in organizing the company's various analytical departments, both plant and research, into a coordinated group. This was effected by holding conferences for the demonstration and application of the newer instrumental methods to analytical problems in research and plant control. While at Eastman, apparatus for automatic microcombustions was designed and perfected.

An opportunity to pursue further the path started at Eastman came with the organization of a Research Laboratory at Easton, Pa., by General Aniline and Film Corporation. Early in 1943 he was given the responsibility of organizing the analytical chemistry of the new laboratory and now is actively engaged in developing and applying the newer techniques of microchemistry and instrumental methods to the research and plant problems of this company.

Dr. Hallett was born November 7, 1900, in Oakland, Calif., and received his B.A. in 1923 from the University of British Columbia. He was secretary of the Microchemical Section of the AMERICAN CHEMICAL SOCIETY in 1937, chairman in 1939, and served on several analytical committees during the inter-



Lawrence T. Hallett

vening years. Publications include articles on solubilities, microtechniques, and a review on organic microchemistry. He contributed the section on microanalysis in Scott's "Standard Methods of Chemical Analysis".

Ray Parkin Chapman, in charge of the central analytical laboratory in the Stamford Research Laboratories, American Cyanamid Company, was born in the Province of Prince Edward Island, Canada, July 15, 1898. He received his A.B. degree from Mount Allison University, Sackville, New Brunswick, Canada, in 1921, after having taken time out from college for two years' service with the Canadian Expeditionary Force during World War I. He taught chemistry and mathematics in high school for several years, meanwhile attending summer sessions at Columbia University (Teachers College) and receiving his M.A. degree in 1928. He entered Columbia University as a graduate student in the Department of Chemistry in 1929 and received his Ph.D. degree in 1932, serving during this period first as assistant in chemistry at Columbia and later as instructor at St. Stephen's College. His research for the doctorate dealt with indicators for oxidation-reduction reactions and resulted in the discovery of the indicator properties of the now familiar ferro-o-phenanthroline complex ion.

After a year as research assistant at Columbia he joined the Experimental Laboratory of the American Cyanamid Company at Linden, N. J., in 1933. He has been in charge of the central analytical laboratory of that company's Stamford Research Laboratories since its organization in 1937.

He has been a member of the AMERICAN CHEMICAL SOCIETY since 1931, and is a member also of various committees of the American Society for Testing Materials and of Sigma Xi and Phi Lambda Upsilon.

J. Raynor Churchill was born in Denver, Colo., in 1911 and went to Pittsburgh in 1919, when his father, H. V. Churchill, became chief chemist of the Aluminum Company of America. Intending to become a mechanical engineer, he worked for a few months in the Machine Shops of the Aluminum Company of America, and for a year as a millwright at the Ford Plant in Detroit. Returning to Pittsburgh, he entered the Evening College of Engineering at the Carnegie Institute of Technology. In 1929 he joined the staff of the Aluminum Research Laboratories as laboratory assistant. He completed the 9-year evening

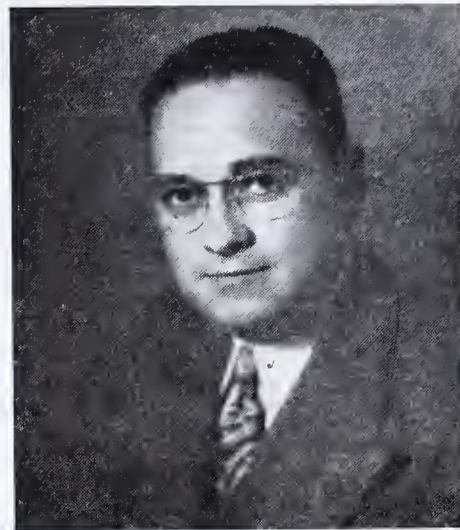




J. Raynor Churchill



Ray P. Chapman



SHELBURNE STUDIOS

Bernard L. Oser

course at Carnegie Tech in 1938, obtaining the bachelor's degree, and took a number of graduate subjects in the evening school during the next few years.

Most of his professional experience has been in the field of spectrography from the time the first modern spectrograph was obtained by the Aluminum Company of America in 1931. He became chief spectrographer in 1941 and has been in charge of the company's spectrographic operations since that time. He has had an important part in the tremendous expansion of its analytical facilities during the past few years. The company is now relying on the bulk of its metallurgical analysis by means of the spectrograph and is using 40 spectrographs in 31 laboratories distributed over the country. Most of his time during the past few years has been spent on the development of equipment and techniques for spectrographic analysis and in the technical supervision of spectrographic laboratories. He has done some research in allied fields and has been the author or co-author of papers on spectrography, electrographic methods of analysis, and corrosion phenomena.

Bernard L. Oser was born in Philadelphia, February 2, 1899. He received the B.S. in chemistry from the University of Pennsylvania in 1920, working under Edgar Fahs Smith, and the M.S. in 1925, working under D. Wright Wilson; and his Ph.D. in 1927 from Fordham University, working under Carl P. Sherwin. He was employed as chemist in the Dermatological Research

Laboratories from 1918 to 1920, as assistant in the Department of Physiological Chemistry, Jefferson Medical College, from 1920 to 1922, and as instructor in the Department of Chemistry, Graduate School of Medicine, University of Pennsylvania, and biochemist, Philadelphia General Hospital, from 1922 to 1926. In 1926 he joined Philip B. Hawk as assistant director in charge of the biological laboratories of the Food Research Laboratories, Inc.; in 1934 he became director and in 1939 vice president.

He has been a collaborator on all revisions of Hawk and Bergeim's "Practical Physiological Chemistry" since the 8th edition and author of several chapters, including those on blood chemistry and vitamins and deficiency diseases. He is the author or co-author of numerous scientific papers relating principally to vitamin methodology, stabilization, and control in foods, fish liver oils, and pharmaceutical products.

He has been a member of the AMERICAN CHEMICAL SOCIETY since 1927, and is at present a member of the Executive Committee, Division of Agricultural and Food Chemistry. Memberships in other scientific societies include: American Association for the Advancement of Science, American Institute of Chemists, New York Academy of Science, Institute of Food Technologists, Animal Vitamin Research Council, Association of Consulting Chemists and Chemical Engineers, Committee on Valid Certification of the American Standards Association, and American Association of Scientific Workers.

## Standard Samples of Hydrocarbons

Work was begun at the National Bureau of Standards on July 1, 1943, on the preparation of standard samples of hydrocarbons of known high purity for calibrating analytical instruments and apparatus.

Compound	N.B.S. Standard Sample No.	Amount of Impurity, Mole Per Cent	Designation	Volume of Hydrocarbon, Ml.	Kind of Container	Cost per Sample
Pentane	201	0.25 ± 0.10	— 5	5	Plain ampoule, sealed in vacuo	\$3.00
Methylbutane (isopentane)	202	0.13 ± 0.06	— 85	8	Special ampoule, with internal vacuum break-off tip, sealed in vacuo	5.00
Hexane	203	0.24 ± 0.09	— 25	25	Plain ampoule, sealed in vacuo	9.00
Methylpentane	204	0.25 ± 0.10				
Methylpentane	205	a				
2-Dimethylbutane	206	0.12 ± 0.05				
2-Dimethylbutane	207	0.06 ± 0.04				
Methylcyclopentane	208	0.25 ± 0.09				
Cyclohexane	209	0.012 ± 0.007				
Benzene	210	0.05 ± 0.02				
Methylbenzene (toluene)	211	0.04 ± 0.02				
Methylbenzene	212	0.20 ± 0.07				
1-Dimethylbenzene (o-xylene)	213	0.14 ± 0.05				
1-Dimethylbenzene (m-xylene)	214	0.17 ± 0.07				
1-Dimethylbenzene (p-xylene)	215	0.07 ± 0.03				

a Not determined; believed to be the same as for 2-methylpentane.

tus in the research, development, and analytical laboratories of the petroleum, rubber, and allied industries. Only a limited quantity of each hydrocarbon has been prepared and the purity of each has been pushed only to a point that is believed to be amply adequate for the present urgent needs for calibration. Fifteen hydrocarbons are now available under this program (see table).

The cost includes delivery in the United States, Mexico, Canada, Cuba, and United States possessions. For other countries, 50 cents postage must be added for each container, plus 25 cents for insurance or registration of each shipment.

Payment must be made with order. Orders should be addressed to the National Bureau of Standards, Washington 25, D. C., specifying clearly by number and name the hydrocarbons and containers wanted.

## Sensitive Indicator for Volumetric Determination of Boiler Feedwater Alkalinity

The reagents used in preparation of the indicator for volumetric determination of boiler feedwater alkalinity [IND. ENG. CHEM., ANAL. ED., 15, 742 (1943)] are:

1. Alphazurine, National Aniline Division, Allied Chemical & Dye Corp., reagent 205.
2. Methyl red sodium salt, Eastman organic chemicals, No. 1462.

Bureau of Ships  
Navy Department  
Washington, D. C.

HARRY FLEISHER



# NOTES ON ANALYTICAL PROCEDURES

## Textile Finishes and Fiber Identification Stains

BRAHAM NORWICK<sup>1</sup>, Beunit Mills, Cohoes, N. Y.

IT HAS been recognized that dye mixtures such as the Hahn stains and the more versatile Davis and Rynkiewicz modification (1) are not entirely adequate for the identification of treated rayon fibers likely to be encountered in processed fabrics. The presence of added substances in the yarn, as well as the chemical and physical history of the fabric, frequently results in peculiar alterations of test dyeing properties. Under such circumstances, once the yarns are known, anomalous dyeing may serve to determine the finish or the extent to which processing has been carried. When the treatment is known, the shade obtained may be of value in control, once one has established a set of standards; this is illustrated in Table I, which indicates the influence of causticizing upon subsequent test dyeing with the Davis and Rynkiewicz stain.

Table I. Effect of Caustic Treatment upon Subsequent Dyeing

Time Min.	Concentration of Caustic				
	25° C.	1% 50° C.	5% 25° C.	50° C.	25%, 25° C.
Acetate					
0.5	Greenish yellow	Greenish yellow	Greenish yellow	Green	Greenish yellow
1	Greenish yellow	Green	Greenish yellow	Green	Greenish yellow
5	Greenish yellow	Blue	Green		
Viscose					
0.5	Lavender	Lavender	Blue	Violet	Dark blue
1	Lavender	Lavender	Blue	Violet	Dark blue
5	Lavender	Lavender	Violet	Dark blue	Dark blue

In testing samples which have had unknown treatment, one must first consider the primary color components which go to make up the shade obtained. The possible significance of the various colors is not easily limited, but the probabilities, especially when considered along with the particular type of fabric under examination, are indicated in Table II.

Table III indicates how one may make further subdivisions in such a typical treatment as sizing.

The various families of finishes may be distinguished by test dyeing before and after special treatments: enzymes for proteins, 5% sulfuric acid at a boil for urea-formaldehyde resins, alcoholic extraction for cationic softeners.

<sup>1</sup> Present address, Aberdeen Proving Ground, Md.

Table II. Color Components

Blue	Yellow	Red
Cuprammonium	Acetate	Viscose
Cotton	Nylon	Attacked wool
Vinyon	Wool	Aldehyde resins
Sizes	Heavy oiling	
Cationic materials	Melamine	
Attacked viscose	Aldehyde resins	
Attacked acetate		

Table III. Alkali- and Water-Soluble Sizes

Blue	No Color
Starches	Natural gums
Protein adhesives	Natural resins
Cellulose ethers	Alkyd resins
Polyvinyl alcohols	

The fibers based upon cellulose can be treated to give to dyeings in all the available shades.

Cotton, dried with formaldehyde and a trace of acid, moistened with ammonia, and then heated either in an oven or in an organic liquid such as lauryl alcohol, quickly loses its ability to pick up blue and shows first reddish shades and then yellow. Drying cotton with glyoxal gives similar results. The presence of melamine, even if unreacted—that is, in a state where it is extractable with water—as it might be if employed for inhibiting the gelatinizing action of concentrated sulfuric acid upon cotton, causes the latter to pick up yellow. Drying viscose with glyoxal yields a yarn which dyes a pale yellow. The common creaseproof and shrink-resisting urea-formaldehyde finish gives yellow shades: one interesting sample of plain weave spun rayon showed a test dyeing yellow on one face and lavender on the other, due to the use of dry cans contacting and reacting the resin on only one face of the fabric.

Incomplete and uneven desizing, which may be manifested in a variety of ways, such as a slight uneven luster or dullness or variable tear strengths and air permeabilities, can be rapidly detected on known fabrics of nylon, viscose, and acetate, since the common size materials stain a deep, contrasting blue. In such cases, and with mixed fibers, examination of the dyed sample under a low-power microscope may be a source of further information about the condition of the material.

There is a relation between the extent of alteration of test dyeing properties and the effective treatment, but this is generally true only over short ranges, and even there one will find in commercial treatments that interferences uncontrollable by the analyst are of such magnitude that in general only qualitative information can be obtained.

Appreciating this fact, however, for satisfactory qualitative tests, the analyst has considerable leeway, following the Davis and Rynkiewicz staining suggestions.

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## Priority Assistance to Laboratories

Conditions under which priority assistance is given to laboratories were clarified March 6 by the issuance of Preference Rating Order P-43 as amended. Any person who carries on scientific or technological investigation, testing, development, or experimentation in his business is considered to operate a laboratory in buying materials for these purposes, even though he does not have a separate department or organization for such activities.

Priority ratings assigned by the order may be used to get materials for development of products designed primarily for future civilian markets only if such activities will be carried on without diverting any manpower, technical skill, or facilities from war work. Laboratories may not use AA-1 preference rating for activities connected with future civilian needs.

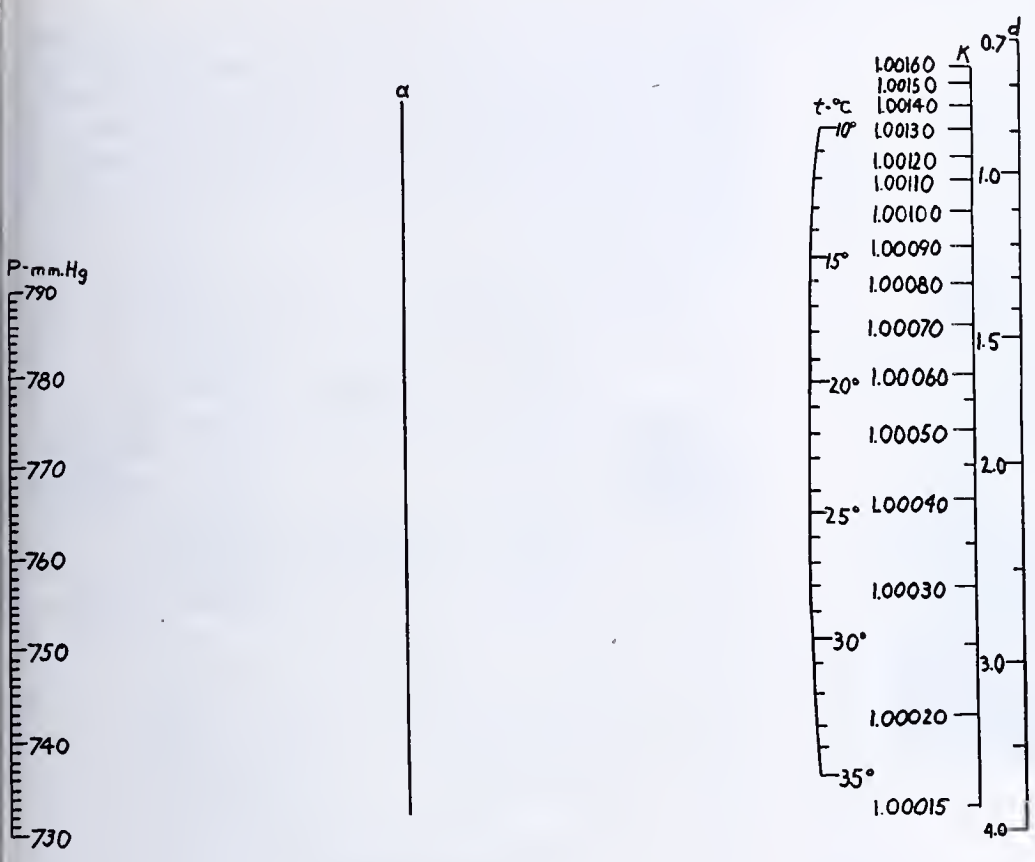
Restrictions on the quantity of aluminum that may be obtained under the order are removed.

Priorities assistance assigned under P-43 may be used for construction jobs costing not more than \$500 without applying for permission to start construction under L-41. Procedure for obtaining controlled materials has been simplified, and the allotment number V-9 is used in place of MRO-P-43.



# Nomographic Chart for Correcting Weights to Vacuum

HENRY C. THACHER, JR., C.W.S. Development Laboratory, Massachusetts Institute of Technology, Cambridge, Mass.



given by  $K = d_m(d_w - d_a)/d_w(d_m - d_a)$ . When  $d_a^2/d_m$  is negligible in comparison with  $d_m$ , this reduces to the commonly used equation,  $K = 1 + d_a(1/d_m - 1/d_w)$ , which has been used in the construction of this chart.

**USE OF THE CHART.** To use the chart, draw a line from the observed Centigrade temperature on scale *t* to the observed pressure on line *P*. From the intersection of this line with line  $\alpha$  draw a second line to the density of the object on line *d*. Then factor *K* will be found at this second line on scale *K*, and the vacuum weight of the object may be obtained by multiplying the observed weight by this factor.

For example, suppose that an object of density 0.70 when weighed in air at 50% relative humidity, 770-mm. barometric pressure, and 20° C., weighs 100.000 grams. Then drawing a line connecting 770 mm. and 20° C., and a second line from 0.70 on the *d* scale to the intersection of the first line with the  $\alpha$  scale, we find *K* equals 1.00158, and the vacuum weight of the object is 100.158.

Or again, when with an object of density 1.5 weighing 10.0000 grams in air at 50 per cent relative humidity with barometer reading 760 mm. and temperature 25° C., *K* is found to be 1.00064, the true weight of the object is 10.0064 grams.

**A**LTHOUGH the correction of weights in air to in vacuo is a very common operation in many fields of precise work, the use of the tables available in handbooks is rather cumbersome when the correction must be applied frequently. Moreover, in many instances greater precision may be required than is obtained by using tables based on constant air density.

The correction factor, *K*, by which the air weight of an object must be multiplied to give its weight in vacuo is a function of the density of the object, the density of the weights used, and the density of the air. The density of the air, in turn, is a function of the barometric pressure, the temperature, and the relative humidity. The effects of pressure and temperature upon the density of the air are relatively great, while the influence of humidity is considerably less, diminishing the effective pressure by 0.3783 times the partial pressure of water vapor.

The accompanying nomograph facilitates the correction of weighings with brass weights to vacuum. All variables except relative humidity of air have been included within the limits indicated below. A constant relative humidity of 50% has been used.

Temperature, 10° to 35° C.  
Barometric pressure, 730 to 790 mm. of mercury  
Density of object, 0.7 to 4.0  
Density of brass weights, 8.4

Inasmuch as the effect of humidity of the air is small, limited variations in relative humidity will have only a slight effect on results. The nomograph therefore offers a more rapid means of computing correction factors and at the same time permits a higher degree of precision, since it is based on constant air humidity rather than on constant air density.

In constructing the chart, it has been assumed that dry air obeys the ideal gas law, and that the effect of humidity upon air density is given by subtracting 0.3783 times the partial pressure of water vapor from the observed barometric pressure. Where  $d_a$  is the density of the air,  $d_m$  is the density of the object, and  $d_w$  is the density of the weights, the vacuum correction factor, *K*, is

The chart may be mounted on a piece of cardboard, and indexes made of loops of thread of suitable length with rubber bands or light springs to give sufficient elasticity. If such loops of thread are placed around the chart, they can be moved at will, and make it unnecessary to draw lines on the chart.

## Joining Plastic Tubing to Glass

RICHARD KIESELBACH, Bakelite Corporation,  
Bound Brook, N. J.

**B**ECAUSE of the present scarcity of rubber and copper, thermoplastic tubing is becoming increasingly popular for laboratory use. Where chemical inertness is desirable, it is far superior to the materials it replaces.

The conventional method of joining plastic tubing to glass involves the use of a short length of rubber tubing. This method is usually satisfactory, where a temporary joint is required. For more permanent connections, or where the use of rubber is objectionable, a neat, strong, vacuum-tight joint can be made in the following manner:

Draw out the end of the glass tubing to a gradual taper, cutting it off at the point where its outside diameter is slightly less than the inside diameter of the plastic tubing. Fire-polish the end and let cool. Then heat the tapered section uniformly in a Bunsen flame for about 2 seconds, and quickly force it into the end of the plastic tubing for a distance of at least 1 cm. (Care must be taken to avoid overheating the glass, since it will then char the plastic.) While it is still hot, press the curled end of the plastic tubing to the glass with the fingers, to give the joint a smoother appearance. Allow the joint to cool thoroughly before putting it into service.

A properly made joint of this kind will be found to be as leak-proof as the tubing itself, and able to withstand a surprising amount of mechanical stress.



# Safety Cap for Laboratory Glass Distillation Equipment

GEORGE A. RADER, Standard Oil Co. of California, Richmond, Calif.

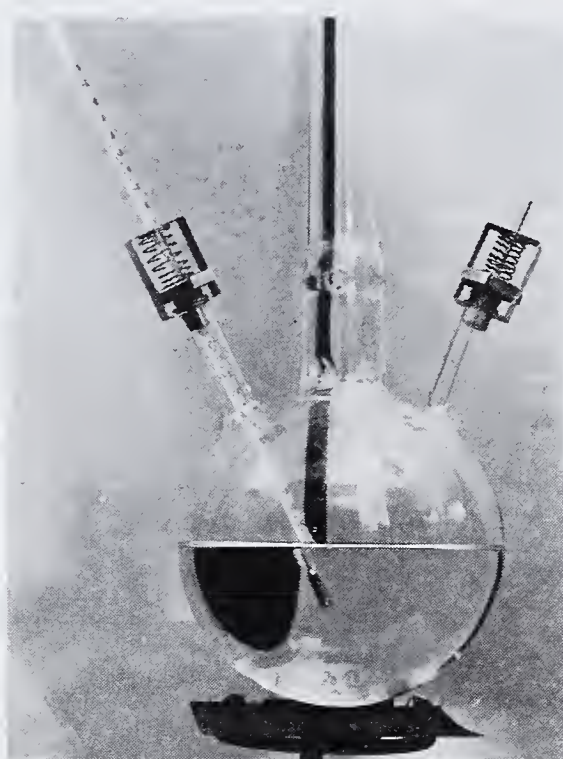


Figure 1

ONE common cause of damaging fires in petroleum laboratories is the accidental building up of excessive pressure in distillation flasks. Such flasks are usually equipped with two stems or necks, one used as the filling stem and the other for a thermometer. Both openings are ordinarily closed with a cork. During the distillation the reflux column or condenser may become plugged, there may be a sudden formation of foam, or the operator may neglect to open a vent line, thereby causing unusual pressure to build up in the flask. When this occurs one cork may be forced out, releasing an appreciable volume of flammable vapor plus liquid which flashes, and the result is a laboratory fire.

A safety device (Figures 1 and 2) has been developed for the purpose of preventing such fires. One device is attached to each stem and acts as a safety valve by permitting the cork to lift

slightly to release the flask pressure, and then to reseal itself. Along with the vapor a small amount of atomized liquid may be released from the flask but the force of the discharge tends to direct the mixture upward and away from the burner flame. If, during the distillation, the flask pressure forces the release of the safety cap, the operator can shut down and correct the cause of pressure build-up.

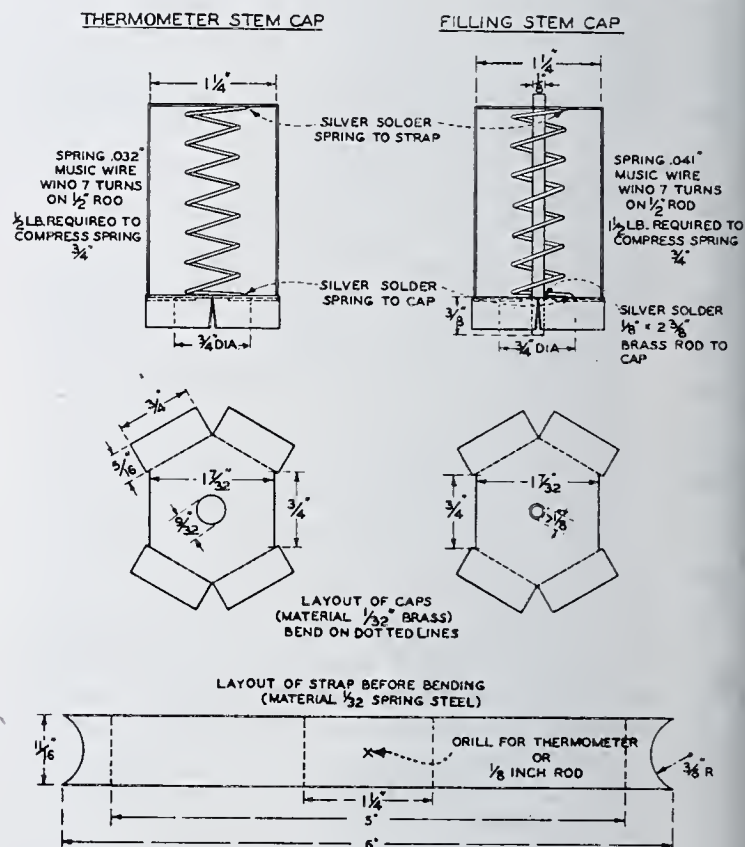


Figure 2

During the tests and demonstrations on laboratory distillation equipment, no flashes or fires resulted, but it is believed that under some conditions a flash is possible. The total amount of stock released, however, is generally so small that serious consequences are not likely to result.

## Improvements in the Determination of Iron by the Nitroso R Salt Method

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THE selection of a new light filter which is more sensitive to the color of the iron salt of 1-nitroso-2-hydroxy-3,6-naphthalene disodium sulfonate and the introduction of certain improvements in the nitroso R salt method for iron (1) have made necessary revision of the original procedure.

**PROCEDURE.** Add to a 10-ml. aliquot of the unknown in a 50-ml. Pyrex test tube, 0.5 ml. of 10% hydroxylamine sulfate and a drop of 0.05% metanil yellow (aqueous solution) and neutralize with 14% ammonium hydroxide drop by drop until a pinkish-yellow color is obtained. (If a decidedly yellow color is produced, add one drop of 6 N hydrochloric acid.) Then add to the mixture 1 ml. of 0.5% nitroso R salt and 2 ml. of 4 N sodium acetate and dilute to a definite volume, in the range of 20 ml. Determine

color intensity in the photoelectric colorimeter from 2 to 24 hours later, using filter KS-66 with transmission limits of 640 to 700 millimicrons and a 2.5-mm. cell or a combination of a 10-mm. cell and a 7.5-mm. plunger as recommended for the Summerson-Klett photoelectric colorimeter. The 10-mm. cell is recommended for concentrations below 1 microgram per ml. A good linear relationship between concentration and colorimetric reading exists up to 10 micrograms of iron per milliliter.

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PUBLISHED with the approval of the Acting Director as Technical Paper No. 151 of the Pineapple Research Institute of Hawaii, University of Hawaii.



# Some Color Tests for Rotenone Not Specific

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OF THE several colorimetric tests proposed for the detection of rotenone (9), the blue color, or Durham, test (4) and the red color, or Gross-Smith-Goodhue, test (5, 6) have been found especially useful. The Durham test is based on the observation that when rotenone is treated with nitric acid and then with ammonia an evanescent blue color is produced; it has been modified by Jones and Smith (11) to make it more delicate and suitable for general use. In the Gross-Smith-Goodhue test a red color is obtained when an alcoholic potassium hydroxide solution containing sodium nitrite is added to rotenone and the mixture subsequently is acidified with sulfuric acid.

Either test can be relied upon to show the presence of rotenone or some of the rotenoids (13) in specimens of *Derris*, *Lonchocarpus*, and *Tephrosia*. The blue color test has been used by Jones *et al.* (10) and by Sievers and associates (14) to select specimens of devil's-shoestring (*Tephrosia virginiana*) highest in rotenone content. Jones *et al.* (10) found that the effectiveness against houseflies of acetone extracts of various species of *Tephrosia* is well correlated with the degree of blue or blue-green color given by the Durham test. By this simple test the effectiveness of a sample of *Tephrosia* can be roughly predicted. Likewise, the red color test has been widely used in the quantitative evaluation of material containing rotenone and some of the rotenoids. Cahn *et al.* (3) found the method useful in an extensive study of the composition of derris root.

These color tests, however, are not always specific for rotenone in other genera of Leguminosae. For example, in 1937 Moore (12), searching for a domestic source of rotenone, reported its presence in *Amorpha fruticosa* because it gave a positive Durham test. Subsequently Featherly (2), of Oklahoma Agricultural and Mechanical College, confirmed the observations of Moore and proposed that seed of the plant be used as a source of rotenone during the war emergency. More recently, however, Acree, Jacobson, and Haller (1) have shown that rotenone is not present in the seeds of *A. fruticosa* and that the blue color is produced by a glycoside whose value as an insecticide remains to be determined.

The yam bean (*Pachyrhizus erosus*) also was reported to contain rotenone solely on the basis of the Durham test (8). Both this plant and *Amorpha fruticosa* give a positive red color test. Certain synthetic organic compounds have also been shown to produce an evanescent blue color when the Durham test is applied (7).

From the foregoing results it appears that considerable caution should be taken in interpreting the color obtained in both these tests when they are applied to plant material other than *Derris*, *Lonchocarpus*, and *Tephrosia*. Rotenone should be reported as present in plants only when it has been definitely isolated and characterized.

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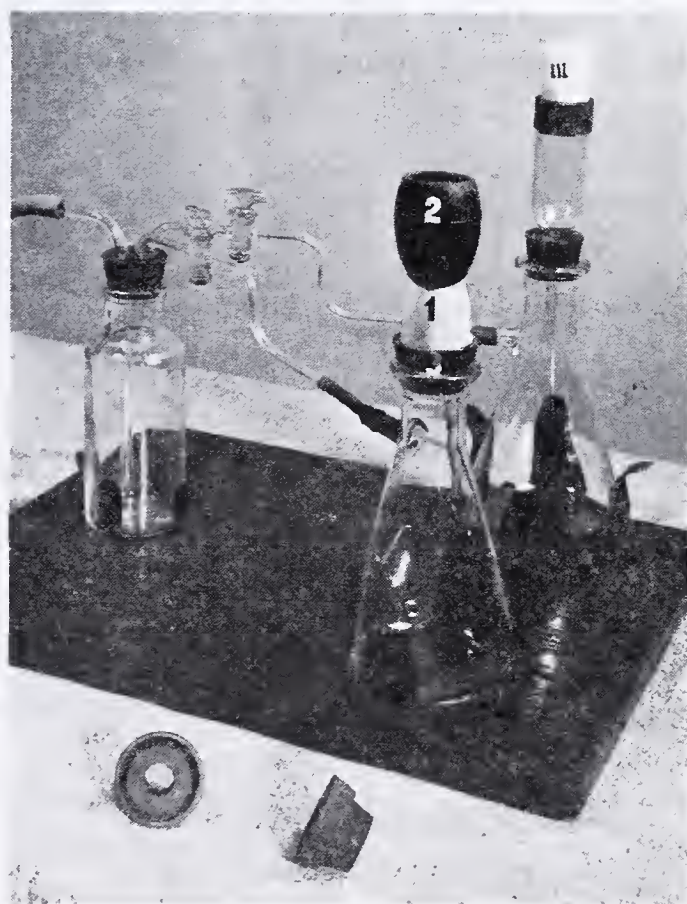
## Washing Selas Filtering Crucibles by Reverse Flow

ERWIN J. BENNE

Michigan Agricultural Experiment Station, East Lansing, Mich.

SINCE it has become impossible to obtain asbestos for analytical filtrations as desirable as that formerly available, the author and his associates have sought equally effective and convenient means of quantitative filtration without the use of asbestos. Selas filtering crucibles meet these requirements for certain determinations, including potassium by the chloroplatinate method, sugar by use of Fehling's solution, and others.

These crucibles are similar to the Gooch type in shape but have a fixed, porous bottom for the filtering element, thereby eliminating the use of asbestos. Directions for cleaning recommend washing with hot water or hot acids by reverse flow in order to remove fine, insoluble particles lodged in the pores of the upper surface of the filter, but do not suggest a convenient, mechanical means of accomplishing this; hence, the author devised the ar-





rangement shown in the accompanying figure to facilitate the task.

1 is a Selas filtering crucible in position for being washed by reverse flow. 2 is a reservoir for the wash solution. It is arranged to fit tightly around the bottom of the crucible being washed and can be easily and quickly transferred from one crucible to another. This exchangeable reservoir was prepared from a large rubber pipet bulb by cutting off the top with a pair of shears and making a hole in the bottom with a cork borer. This hole must be of such size that the bulb can be slipped over the end of the crucible and will fit snugly enough to retain the wash liquid. Used bulbs, deteriorated at the point where they were stretched over glass tubing, may be utilized for such reservoirs, in keeping with the rubber economy program. A short length of Gooch rubber tubing would serve as a satisfactory substitute for such a bulb.

3, prepared from a No. 9 rubber stopper, holds the crucible safely in position for the effective application of suction. A circular groove in the top of the stopper accommodates the upper rim of the inverted crucible, and if filled with water before insertion of the crucible, it provides an effective seal against entrance of air when suction is applied. A large hole in the center of this holder permits exit of the wash liquid from the crucible into the suction flask. In order to use a stopper with a top larger than the mouth of these crucibles, it was necessary to reduce and taper the lower part to fit the neck of the suction flask. Front and side views of these holders are shown in the lower part of the picture. The hole in the center of the stopper was cut with a cork borer in the usual way; the groove in the top was made, and the lower part reduced, by use of a metal-cutting lathe.

These devices used in combination with the arrangement shown for holding the suction flasks and trap bottle provide a convenient means for washing Selas crucibles by reverse flow. After a crucible is washed several times in this way, it is placed in the ordinary crucible holder shown in the suction flask at the right for washing in the usual direction.

#### ACKNOWLEDGMENT

The author is indebted to Wm. Wallace, mechanic in the Department of Agricultural Engineering at Michigan State College, for operating the lathe in the preparation of a number of these crucible holders.

## A.S.T.M. Holds 1944 Committee Week in Cincinnati, February 28 to March 4

In attendance at the 148 technical committee meetings held during the 1944 Committee Week in Cincinnati, February 28 through March 4, were 745 technologists, this figure being almost double that of the previous year, but the registration figure varies, depending on number of standing committees which participate in the group meetings.

Decision by the A.S.T.M. Executive Committee to issue its widely used Book of Standards in 1944 instead of 1945, the normal triennial year, led many of the committees to meet this year in March to get their specifications and other work as up to date as possible. Decision to advance the Book of Standards' publication by one year is caused by the unprecedented demand for the publication.

A number of major A.S.T.M. committees also held meetings immediately prior to or subsequent to Committee Week.

There are constantly increasing recognition and use of A.S.T.M. purchase specifications, methods of tests, and related standards. This is indicated by the demands for publications, the request from industry and government that technical committees extend their work into fields not covered, and also the organization of new committees, such as those on Adhesives, Metal Powders, and Aromatic Hydrocarbons.

The following main A.S.T.M. standing committees, except as noted, met in Cincinnati (usually there were numerous subcommittees and section meetings also):

- |                                    |   |
|------------------------------------|---|
| A-1 on Steel                       | A-10 on Iron-Chromium, Iron-Chromium Nickel, and Related Alloys |
| A-3 on Cast Iron                   | B-3 on Corrosion of Non-Ferrous Metals and Alloys               |
| A-5 on Corrosion of Iron and Steel | B-5 on Copper and Copper Alloys                                 |
| A-6 on Magnetic Properties         |   |
| A-7 on Malleable Iron Castings     |   |

- B-6 on Die-Cast Metals and Alloys
- B-7 on Light Metals and Alloys
- B-8 on Electrodeposited Metallic Coatings
- C-16 on Thermal Insulating Materials
- D-1 on Paint, Varnish, and Related Products
- D-2 on Petroleum Products and Lubricants

- D-4 on Road and Paving Materials
- D-5 on Coal and Coke
- D-9 on Electrical Insulating Materials (Philadelphia, Pa., Feb. 21, 22)
- D-11 on Rubber Products
- D-17 on Naval Stores
- D-20 on Plastics (Philadelphia, Pa., Feb. 23, 24)

## NEW EQUIPMENT

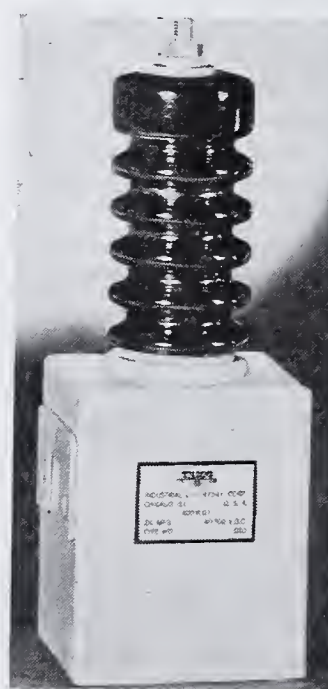
### Instrument for Measuring Small Particles

The Fisher sub-sieve sizer, for measuring the size of particles too small to be measured by sieves, is based on the apparatus described by Gooden and Smith [IND. ENG. CHEM., ANAL. ED., 12, 479 (1940)], and is made by Eimer & Amend, 635 Greenwich St., New York, N. Y. Operation has been simplified and a calculator chart provided to make operation easy. The instrument consists of an air pump, air-pressure regulating device, precision bore sample tube, double range flowmeter, calculator chart, and accessory equipment.

In operation, the motor-driven air pump builds up pressure in the pressure regulator to a constant head, so that a uniform flow of dry air passes through the packed powder sample and is measured by means of a double range flowmeter. The liquid level in the manometer varies with the air flow, depending on the resistance (particle size) of the particular sample. The average particle size of the sample in microns is read directly from the chart as indicated by the level of the fluid in the manometer. The range of the instrument is from 0.2 to 50 microns.

### Capacitor for Electron Microscope

A 0.01-mfd. 40,000-volt direct current capacitor has been built for a special application of the electron microscope. It is capable of continuous operation at 80° C. and of withstanding total submersion and heavy surges. The case is welded steel measuring  $4\frac{11}{16} \times 5\frac{3}{4} \times 7$  inches high with a standoff insulator 8.5 inches high, soldered directly to the case, thus eliminating the possibility of oil leakage or the entrance of moisture. It is manufactured by the Industrial Specialty Co., 1725 West North Ave., Chicago 22, Ill.



### Lead Standard

The American Standards Association has announced approval of a new standard, Allowable Concentration of Lead and Certain Inorganic Compounds (Z37.11-1943), which sets safe limits for the amount of metallic lead, lead carbonate, lead sulfate, lead oxides, lead nitrate, and lead chloride allowable in the air of industrial workshops. It covers the physical and chemical properties of the substance, summarizes methods of test, and includes a comprehensive bibliography.

Copies may be obtained from the American Standards Association, 29 West 39th St., New York 18, N. Y., at 20 cents each.



# BOOK REVIEWS

**Semimicro Quantitative Organic Analysis.** *E. P. Clark.* 1st ed. 135 pages. Academic Press, Inc., 125 East 23rd St., New York, N. Y., 1943. Price, \$2.50.

The book was written for the purpose of providing laboratories, which are not equipped for precision microanalysis, with directions for analysis on a semimicro scale with samples ranging from 10 to 25 mg. It is excellent in this respect and fills a great need. However, the book fails to give a reasonable number of alternative methods or even a bibliography of such, and includes very few references to the literature dealing with the methods recommended. The author tries to justify this condition by stating in the preface that he did not attempt "to compile a reference book but rather to present in as brief a manner as possible simple working, well tested methods, which can be followed to a successful conclusion". Procedures are described which make use of microchemical apparatus, but it is unfortunate that the author, in his setups, has ignored the specifications of the AMERICAN CHEMICAL SOCIETY Committee on Standardization of Microchemical Apparatus, since all large glass blowers and scientific houses now have these. In many cases the apparatus described practically meets the specifications but they should have been complied with throughout. The reader will do well to use standard parts for all setups, except for the semimicroazotometer, which is much larger.

The book is divided into 13 chapters. Unfortunately, determinations of ash and metals have not been included.

**CHAPTER I. INTRODUCTION.** The use of a semimicrobalance is recommended and a table is included showing the relationship between the precision of a balance and the practical size of a sample to be weighed. At the top of page 3 the essential difference between precision and sensitivity does not seem to be appreciated. Temperature effects, lighting arrangements, care of the balance, calibration of the weights, and weighing are discussed, with directions for making various standard volumetric solutions for semimicro work. Filtering and drying apparatus are described and there are directions for determination of melting and boiling points. Some space is devoted to calculation of empirical formulas from analytical data.

**CHAPTER II. DETERMINATION OF CARBON AND HYDROGEN.** The procedure is essentially the Pregl setup using micro-sized equipment. Lead chromate has been omitted from the standard tube filling and a preheater is employed. Approximately 10-mg. samples are burned in an atmosphere of oxygen at a temperature of about 550° C. for all samples. Persons using this book as a guide for their carbon-hydrogen determinations will do well to increase the temperature of their furnaces to about 680° C., as 550° C. is much too low for many classes of compounds. The author does not recommend a second combustion, which is also a rather dangerous procedure since many compounds have a tendency to sublime back; the author attempts to prevent this by the use of a platinum baffle. The specifications for the preheater, bubble counter, combustion tubes, absorption tubes, and Mariotte flask are not standard.

**CHAPTER III. DETERMINATION OF NITROGEN BY THE KJELDAHL METHOD.** The author greatly favors the Kjeldahl method over the Dumas for practically all types of compounds, stating that only in the case of certain semicarbazones is the method not applicable. Included in his discussion is the Friedrich method for handling compounds containing N-N, NO, and NO<sub>2</sub>. Diagrams for constructing an electric digester and the Parnass-Wagner electrically heated distillation apparatus are included.

**CHAPTER IV. DUMAS METHOD FOR DETERMINATION OF NITROGEN.** A semimicro setup is described which uses microcombustion tubes, and an azotometer of 5-cc. capacity constructed from a microburet. There is no three-way stopcock or other form of regulator between the combustion tube and the azotometer. Several carbon dioxide generators are described which give a good grade of gas. The author suggests that combustions here be done at a "dull red heat" and merely employs one combustion. It would be far better to use a temperature of about 650° to 680° C. and give the tube a second combustion following the procedure of Pregl.

**CHAPTER V. DETERMINATION OF HALOGENS.** The ethanolamine-sodium method is presented as the one of greatest importance; following this is a description of the Carius method using 25-mg. samples with details for constructing the semimicro Carius furnace. Next is a description of the sodium peroxide fusion method, giving

details for both gravimetric and volumetric procedures. The fusion mixture described on page 58 should not be prepared and kept as suggested, because of its explosive nature. For determination of iodine the Liepert volumetric method is stressed, and finally a method for displacing aliphatic iodine with bromine followed by titration of the iodine formed. The filter tubes are not standard. The catalytic combustion method for halogens should have been included.

**CHAPTER VI. DETERMINATION OF SULFUR.** The author describes the Carius determination using 25-mg. samples and heating the bomb tubes to 300° C. This temperature is in disagreement with that recommended by the standard texts on microchemistry, which advise keeping the temperature below 270° C. because of the danger of fusion of barium sulfate in the glass. The analyst will do well to use the lower temperatures. The method described for filtering is not micro in nature but rather macro, since it suggests transferring the barium sulfate to a Gooch crucible with the aid of a stirring rod. The reader will do well to use the methods generally employed for microdetermination of sulfur, either the inverted filter method or the Neubauer crucible. As in the chapter on halogens, the catalytic combustion method should have been included.

**CHAPTER VII. DETERMINATION OF PHOSPHORUS.** The alkalinic nitrate fusion method for converting organic phosphorus to orthophosphate, followed by conversion of phosphorus to phosphomolybdic anhydride according to Woy's procedure, is given. The Kjeldahl and sodium peroxide methods are mentioned but not recommended, although they are known to give excellent results.

**CHAPTER VIII. DETERMINATION OF METHOXYL AND ETHOXYL GROUPS.** A semimicro alkoxyl apparatus is described and a slightly modified Vieböck and Schwappach volumetric procedure used. The setup is certain to give low results with such compounds as methyl esters, which split off methyl alcohol almost immediately upon contact with the hydriodic acid, unless the reaction mixture is allowed to stand at room temperature for about a half an hour before heating of the hydriodic acid is begun. The analyst would do well to modify the setup to include a reflux condenser between the boiling flask and the trap, while the latter should contain sodium thiosulfate and cadmium sulfate in place of water, as the author recommends. Valuable information is given regarding the preparation of acid suitable for the determination, as even the so-called reagent grade of hydriodic acid recommended for microanalysis gives tremendous blanks.

**CHAPTER IX. DETERMINATION OF ACETYL GROUPS.** The author recommends hydrolysis of the acetyl compounds with ethanolic or *N*-butanolic potassium hydroxide for *O*-acetyl or *N*-acetyl compounds respectively, followed by acidification, distillation, and titration of the liberated acetic acids. This method was chosen in preference to the very excellent one of hydrolysis with *p*-toluene sulfonic acid followed by vacuum distillation of the acetic acid. The reader will do well to use the latter method of Elek and Harte or at least read the earlier articles by Clark, referred to in the footnotes, before trying his method.

**CHAPTER X. DETERMINATION OF THE NEUTRALIZATION EQUIVALENT.** A procedure for determining the neutralization equivalent of compounds is given, together with some valuable suggestions in the so-called notes.

**CHAPTER XI. DETERMINATION OF MOLECULAR WEIGHTS.** The Signer method of isothermal distillation and the Rast method of freezing point lowering are presented, with greater emphasis upon the former method. The author describes an apparatus for use with the isothermal distillation method. No reference is made to the work of Niederl and his collaborators which the reader will do well to review.

**CHAPTER XII. DETERMINATION OF VOLATILE FATTY ACIDS.** The author describes in detail a method for qualitatively and quantitatively determining volatile fatty acids in dilute solutions, chiefly based on his own work. Examples are given as to the usefulness of this method. The earlier papers referred to in the footnotes should be read before attempting this work.

**CHAPTER XIII. SOME USEFUL TABLES.** There are included tables of gravimetric factors, barometer corrections for temperature, atomic and molecular formulas and some of their multiples, the carbon and hydrogen percentages and molecular weights of a series of CHO compounds from C<sub>15</sub> to C<sub>32</sub> frequently encountered among natural products and their derivatives, and a five-place table of logarithms of numbers from 1 to 10,000. A rather complete index follows at the end.

AL STEYERMARK



**Laboratory Manual of Spot Tests.** *Fritz Feigl.* Translated from German manuscript by *Ralph E. Oesper.* 276 pages. Academic Press, Inc., 125 East 23rd St., New York 10, N. Y., 1943. Price, \$3.90.

This book was consciously designed by its author for the teaching of advanced chemistry in general, and of spot-test analysis in particular. The reviewer deliberately lists advanced chemistry as the subject of primary pedagogical interest, despite the book's title. Thus are marked two departures for Professor Feigl: whereas his former works were monographs for the laboratory worker in the specialized field of spot analysis, this volume is intended primarily for the student who is learning, not spot testing or even analysis, but advanced chemistry in general.

This is an interesting idea. The author's argument points out that college courses in classical qualitative analysis are offered not so much for the purpose of producing chemical analysts, but as a framework, interesting and useful in itself, on which to hang much of the body of fact and theory that constitutes the essentials of chemical science. The argument submits simply that spot testing itself is sufficiently broad in its foundations, and tortuous in its ramifications into the bases of chemistry, to justify its use as a similar framework. The reviewer is not a teacher, and must leave to teachers the authoritative judgment on the validity of this argument; but he hopes that Feigl's unique idea, and this excellent book that is its medium, will be accorded their sympathetic attention. "Extremely important teaching goals", the author writes, "are: the closest possible correlation between handiwork (*sic*) and knowledge; the development of a critical sense; the acquisition of the ability to discern the relations of individual observations and findings to each other." If this book facilitates the achievement of these goals, its worth will have been demonstrated.

The reviewer need add nothing to the author's summary of the book's contents:

The Manual opens with a general discussion of the theoretical foundations of the subject. Then follows a chapter on the technique of spot testing, including a description of the necessary equipment. Next comes a chapter, in six parts, giving an extended treatment of surface and capillary effects, which are of such great importance in spot reactions. Appropriate instructive experiments are described. The following chapter deals with spot reactions designed to detect or identify inorganic materials. . . . The next chapter, on Qualitative Organic Spot Analyses, consists of three parts: detection of certain elements in organic compounds, detection of certain characteristic groups of atoms, detection of certain compounds. This arrangement accords best with the fundamentally different types of problems encountered in qualitative organic analysis. The three succeeding chapters deal respectively with the practical application of spot reactions to the testing of rocks and minerals, industrial materials, biological substances. Numerous practical examples are given in each of these chapters. The Manual closes with a chapter on Quantitative Determinations by Means of Spot Colorimetry.

Much of the material in this book will of course be found, differently presented, in the author's previously published "Spot Tests"; but there is a considerable amount of new material, too. The reviewer is particularly pleased to see the inclusion of the three chapters on the practical testing of rocks and minerals, industrial materials, and biological substances.

The book has an attractive appearance. No typographical errors were noticed.

BEVERLY L. CLARKE

**Examination of Waters and Water Supplies.** *Ernest Victor Suckling.* 5th ed. 849 pages. Blakiston's Son & Co., Philadelphia, Pa. Price, \$12.00.

This is the latest edition of the original text which was first issued by J. C. Thresh in 1904 and has been a standard textbook on water supplies and their examination since that time. The present edition is of broader scope than the fourth, published in 1933, and contains valuable information on water treatment, primarily from a sanitary viewpoint.

This revised, expanded, and modernized text is divided into eight parts, with several chapters under each section. The arrangement and selection of the material are excellent and, like the former editions, the book is very well written. The subject is presented largely

from the viewpoint of British requirements, but the author has referred to recent practices in this country and has drawn extensively from articles and papers in technical journals and similar publications in the United States. These references have been selected with care and will be helpful to those wishing to refer to the origin of a great deal of the data presented. The book will be found informative to scientists in this country who are interested in certain phases of water-borne diseases, since there is much material in it which is not readily available in American texts.

The analytical data and illustrations, especially those relating to microscopic organisms, have been very well prepared. The book will be a valuable addition to any sanitarian's library, and its purchase is especially justified as a reference work.

S. T. POWELL

**Standard Methods for Testing Petroleum and Its Products.** 5th ed. 500 pages, 127 diagrams. Institute of Petroleum, London, England, and American Society for Testing Materials, 260 South Broad St., Philadelphia, Pa., 1944. Price, \$3.00.

This new edition of The Institute of Petroleum's handbook contains details of 97 methods for testing petroleum and its products.

New matter includes an additional method for determining aniline points of volatile materials, a low-temperature filtration test for gas oil and Diesel fuels, the Fraass breaking point test for bitumen, a congealing point test for waxes for which the setting point method is unsuitable, a method for determining the water tolerance of a motor fuel, new distillation tests for crude oil and residues, a method for estimating the salt content of crude oil, and the use of the Abel flash point apparatus for viscous petroleum mixtures and of the N.G.A.A. metal pycnometer for determining the specific gravity of liquefied gases. A.S.T.M. methods, with slight modification, have been adopted for determining the tetraethyllead content of motor fuel and the residue on evaporation of kerosene and tractor fuels.

An innovation is inclusion of methods for testing bituminous emulsions and chemicals derived from petroleum. The sampling methods have been completely revised, and major alterations have been made in methods for acidity, ash content, Ramsbottom carbon residue, cold test of motor fuels, color (Lovibond), dielectric strength, doctor test, drop point, free acid and alkali in grease, knock rating, oxidation of lubricating oils, oxidation (gum) stability of motor fuels, penetration, sludging value, softening point, Reid vapor pressure, and kinematic viscosity.

Specifications for wartime hydrometers and thermometers are included, and the Appendix has been enlarged by the addition of a standard scheme for evaluating crude oils and a vapor pressure/temperature nomograph for hydrocarbons.

## Directory of Textile Testing Laboratories

The Directory of Commercial and Educational Textile Testing Laboratories is being released by the Textile Foundation at this time when test methods and specification requirements are all-important to manufacturers and consumers. Laboratories are listed alphabetically, according to tests which they are equipped to perform, and geographically, in a 20-page pamphlet.

The foundation is particularly anxious to make available a complete and up-to-date directory and would welcome the names of textile testing laboratories not listed, as well as suggestions which would make a revised directory of greater value. Single copies are sold for 25 cents, 5 for \$1.00, by the Textile Foundation, Industrial Building, National Bureau of Standards, Washington 25, D. C.

## Analysis of Coal and Coke

The American Standards Association recently announced approval of a revision of the Standards Methods of Laboratory Sampling and Analysis of Coal and Coke (K18.1-1944), the work of Committee D-5 of the American Society for Testing Materials. The standard has been widely used since it was adopted in 1927, and has been revised several times previously. It may be obtained for 25 cents from the American Standards Association, 29 West 39th St., New York 18, N. Y.



## Recommended Specifications for Analytical Reagent Chemicals

Acid Hydriodic with Preservative, Acid Perchloric, Dimethylglyoxime, Lead Subacetate (for Sugar Analysis), Manganese Sulfate Monohydrate, Mercuric Oxide Yellow, Phosphorus Pentoxide, Sodium Phosphate Dibasic Heptahydrate, Zinc

EDWARD WICHERS, A. Q. BUTLER, W. D. COLLINS, P. H. MESSINGER, R. A. OSBORN, JOSEPH ROSIN, AND J. F. ROSS  
Committee on Analytical Reagents, AMERICAN CHEMICAL SOCIETY

THE specifications given below comprise the thirteenth group to be published by the committee since 1925, and the first to supplement and amend those appearing in the twelve earlier publications (1-12), which were contained in the book, "A.C.S. Analytical Reagents", published in 1941.

The specifications are intended to serve for reagents to be used in careful analytical work. The limits and tests are based on published work, on the experience of members of the committee in the examination of reagent chemicals on the market, and on studies of the tests made by members of the committee as the various items were considered. Suggestions for the improvement of the specifications will be welcomed by the committee.

In all the directions the acids and ammonium hydroxide referred to are of full strength unless dilution is specified; dilution indicated as (1 + 3) means 1 volume of the reagent or strong solution with 3 volumes of water; "water" means distilled water of a grade suitable for the test described; reagents used in making the tests are supposed to be of the grade recommended below or in previous publications (1-12) from the committee. Directions for the preparation of the ammonium molybdate solution are given under the test for phosphate in ammonium nitrate (3). A time of 5 minutes is to be allowed for the appearance of precipitates and before observation of color reactions, unless some other time is specified.

Blank tests must be made on water and all reagents used in the tests unless the directions provide for elimination of errors due to impurities. Solutions of samples must be filtered for tests in which insoluble matter would interfere.

### Acid, Hydriodic, with Preservative

Replacing previously published specification for Acid, Hydriodic (12).

NOTE. To avoid danger of explosions this acid should be distilled only in an inert atmosphere.

#### Requirements

ASSAY (HI). Not less than 47.0%.  
CHLORIDE AND BROMIDE (as Cl). Not more than 0.1%.  
SULFATE (SO<sub>4</sub>). Not more than 0.005%.  
ARSENIC (As). Not more than 0.0005%.  
PRESERVATIVE (H<sub>3</sub>PO<sub>3</sub>). Not more than 1.5%.  
HEAVY METALS (as Pb). Not more than 0.001%.  
IRON (Fe). Not more than 0.001%.

#### Tests

ASSAY. Weigh about 50 cc. of water in a 250-cc. glass-stoppered flask; add 0.7 cc. of the acid and weigh again. Add 50 cc. of 0.1 N silver nitrate solution, and shake the mixture well. Then add 5 cc. of nitric acid and heat the mixture on the steam bath until the precipitate has acquired a bright yellow color. Cool, and titrate the residual silver nitrate with 0.1 N ammonium thiocyanate solution, using ferric ammonium sulfate as the indicator.

CHLORIDE AND BROMIDE. Dilute 1.2 cc. to 100 cc. and take aliquots of 1 cc. and 5 cc. To the 1-cc. aliquot add 0.08 mg. of chloride. Dilute each aliquot to 20 cc., add 1 cc. of ammonium hydroxide, and then slowly, with vigorous stirring, add 2 cc. of a 5% solution of silver nitrate. Heat to boiling for 5 minutes and stir thoroughly. Cool, shake well, and filter. To the filtrate add nitric acid until neutral and then 1 cc. in excess. The turbidity in the aliquot of 5 cc. should not be greater than the turbidity in the 1-cc. portion, to which 0.08 mg. of chloride was added.

SULFATE. Dilute 3 cc. of the acid with 45 cc. of water, neutralize with ammonium hydroxide, and add 1 cc. of hydrochloric acid. Heat to boiling, add 3 cc. of a 10% solution of barium chloride, and allow to stand overnight. If any precipitate is formed, filter, wash thoroughly, ignite, and weigh. The weight of the ignited precipitate should not be more than 0.0006 gram greater than the weight of the ignited precipitate from a blank made with the quantities of reagents used in the test, including filtration and ignition.

ARSENIC. Dilute 0.4 gram with 10 cc. of water, add 1 cc. of nitric acid, and evaporate on the steam bath to expel the iodine. Determine the arsenic in the residue by the modified Gutzeit method. Any stain produced should not exceed that produced by 0.002 mg. of arsenic.

PRESERVATIVE. Weigh about 2 cc., dilute to 20 cc., add 15 cc. of 30% hydrogen peroxide, and allow to stand for 15 minutes. Heat the solution on the steam bath until all the iodine is volatilized and the solution is colorless. Add 50 cc. of water, 1 gram of ammonium chloride, and 15 cc. of magnesia mixture (5.5 grams of magnesium chloride, 7 grams of ammonium chloride, and 13 cc. of ammonium hydroxide per 100 cc. of solution), and allow the precipitate to settle for 10 minutes. Add 40 cc. of diluted ammonium hydroxide (2 + 3), stir for 10 minutes, and allow to stand at room temperature for 4 hours. Filter, and wash the precipitate well with diluted ammonium hydroxide (1 + 19). Dry the residue and ignite it to constant weight. The weight of H<sub>3</sub>PO<sub>3</sub> calculated from the weight of magnesium pyrophosphate obtained (factor 0.593) should not be more than 1.5% of the weight of the sample taken for the test.

HEAVY METALS. To 1.2 cc. add 3 cc. of sulfuric acid and heat to volatilize the iodine. Add 20 cc. of water, neutralize with ammonium hydroxide, and add 1 cc. of 0.1 N hydrochloric acid. Dilute the solution to 50 cc. and pass hydrogen sulfide through



the solution. Any brown color should not be darker than is produced by 0.02 mg. of lead in an equal volume of solution containing the quantities of reagents used in the test.

**IRON.** Make the solution from the preceding test slightly alkaline with ammonium hydroxide. Any green color should not be more than is produced by 0.02 mg. of iron in an equal volume of solution containing the quantities of reagents used in the test.

### Acid, Perchloric

Replacing previously published specification for Acid, Perchloric 60% (8).

#### Requirements

**ASSAY.** Not less than 70%  $\text{HClO}_4$ .  
**NONVOLATILE MATTER.** Not more than 0.005%.  
**CHLORIDE (Cl).** Not more than 0.001%.  
**NITROGEN COMPOUNDS (as N).** Not more than 0.004%.  
**SULFATE ( $\text{SO}_4$ ).** Not more than 0.005%.  
**AMMONIA ( $\text{NH}_3$ ).** Not more than 0.001%.  
**HEAVY METALS.** To pass test (limit about 0.0005% lead).  
**IRON (Fe).** Not more than 0.0002%.

#### Tests

**ASSAY.** Titrate a weighed portion of the acid, after suitable dilution, with standard alkali hydroxide solution, using phenolphthalein as indicator.

**NONVOLATILE MATTER.** Evaporate 10 cc. of the acid and ignite at low red heat. The weight of the residue should not exceed 0.0007 gram.

**CHLORIDE.** Dilute 2 cc. with 40 cc. of water, add 3 cc. of nitric acid, and 1 cc. of 0.1 *N* silver nitrate. Any turbidity should not be greater than is produced by 0.03 mg. of chloride ion in an equal volume of solution containing the quantities of reagents used in the test.

**NITROGEN COMPOUNDS.** Dilute 1 cc. of the sample in a flask with 10 cc. of ammonia-free water, add 10 cc. of 10% sodium hydroxide solution, and about 0.5 gram of aluminum wire in small pieces, and allow to stand for 3 hours protected from loss or absorption of ammonia. Dilute to 50 cc., decant from any insoluble matter, and add 2 cc. of Nessler's reagent. The color should not be greater than is produced by 0.06 mg. of nitrogen as  $\text{NH}_3$  in the same volume of a solution containing the quantities of sodium hydroxide and Nessler's reagent used in the test.

**SULFATE.** Dilute 10 cc. of the acid with 100 cc. of water and neutralize with ammonium hydroxide, using litmus paper as indicator. Add 0.5 cc. of dilute hydrochloric acid (1 + 9) and 5 cc. of 10% barium chloride solution, and allow to stand overnight. Any precipitate of barium sulfate should not weigh more than 0.0020 gram. Correction should be made for the weight obtained on running a blank, including filtration, using the quantities of reagents used in the test. Most of the ammonia should be removed by evaporation, so that only a small quantity of acid will be required for neutralization before acidifying for the precipitation.

**AMMONIA.** Dilute 2 cc. of the sample with 40 cc. of ammonia-free water, add 10 cc. of 10% sodium hydroxide solution and 2 cc. of Nessler's reagent. Any yellow color produced should not be greater than is given by 0.03 mg. of ammonia in the same volume of a solution containing the quantities of sodium hydroxide and Nessler's reagent used in the test.

**HEAVY METALS, IRON.** Dilute 3 cc. with 40 cc. of water, add 5 cc. of hydrogen sulfide water, and make alkaline with ammonium hydroxide. No brown color should be observed. Any greenish color should not be greater than is produced by 0.01 mg. of iron in an equal volume of alkaline sulfide solution.

### Dimethylglyoxime

#### Requirements

**INSOLUBLE IN ALCOHOL.** Not more than 0.05%.  
**RESIDUE ON IGNITION.** Not more than 0.05%.  
**SUITABILITY FOR NICKEL DETERMINATION.** To pass test.

#### Tests

**INSOLUBLE IN ALCOHOL.** Gently boil 2 grams with 100 cc. of ethyl alcohol under a reflux condenser until no more dissolves. Filter on a tared filtering crucible, wash with 50 cc. of alcohol, and dry at 105–110° C. The weight of the insoluble residue should not exceed 0.0010 gram.

**RESIDUE ON IGNITION.** Ignite 1 gram at a temperature just high enough to burn off the carbonaceous matter. Cool, add 1

drop of sulfuric acid, and continue the ignition at dull redness for 5 minutes. The weight of the residue should not exceed 0.0005 gram.

**SUITABILITY FOR NICKEL DETERMINATION.** Dissolve 1.0 gram of nickel ammonium sulfate hexahydrate in exactly 50 cc. water. Dilute 20 cc. of this solution to 100 cc., heat to boiling and add a solution of 0.25 gram of the dimethylglyoxime in 10 cc. of alcohol. Add diluted ammonium hydroxide (1 + 4), drop by drop, to alkaline reaction, cool, and filter. Add to the filtrate 1 cc. of the nickel ammonium sulfate solution and heat to boiling. A substantial precipitate of nickel dimethylglyoxime should appear.

### Lead Subacetate (for Sugar Analysis)

Replacing specification previously published (10).

#### Requirements

**BASIC LEAD ( $\text{PbO}$ ).** Not less than 33%.  
**INSOLUBLE IN ACETIC ACID.** Not more than 0.05%.  
**INSOLUBLE IN WATER.** Not more than 2.0%.  
**MOISTURE (Loss at 100° C.).** Not more than 1.5%.  
**CHLORIDE (Cl).** Not more than 0.005%.  
**NITRATE ( $\text{NO}_3$ ).** To pass test (limit about 0.003%).  
**SUBSTANCES NOT PRECIPITATED BY HYDROGEN SULFIDE.** Not more than 0.30%.  
**COPPER (Cu).** Not more than 0.005%.  
**IRON (Fe).** Not more than 0.005%.

#### Tests

**BASIC LEAD.** Weigh accurately about 5 grams and dissolve in 100 cc. of carbon dioxide-free water in a 500-cc. volumetric flask. Add 50 cc. of normal acetic acid and 100 cc. of a carbon dioxide-free 3% solution of sodium oxalate. Mix thoroughly, dilute to volume with carbon dioxide-free water, and allow the precipitate to settle. Titrate 100 cc. of the clear supernatant liquid with normal sodium hydroxide, using phenolphthalein as indicator. Each cubic centimeter of normal acetic acid consumed is equivalent to 0.1116 gram of  $\text{PbO}$ .

**INSOLUBLE IN ACETIC ACID.** Dissolve 5 grams in 100 cc. water and 5 cc. of acetic acid, warming if necessary to complete solution. If an insoluble residue remains, filter and wash on the washings are no longer darkened by hydrogen sulfide. Dry at 105–110° C. The weight of the residue should not exceed 0.0025 gram.

**INSOLUBLE IN WATER.** Agitate 1 gram in a small stoppered flask with 50 cc. of carbon dioxide-free water and filter at once. Wash with carbon dioxide-free water and dry at 105–110° C. The weight of the residue should not exceed 0.0200 gram.

**MOISTURE.** Weigh accurately about 0.5 gram and dry for 2 hours at 105–110° C. Cool and reweigh. The loss in weight should not exceed 1.5%.

**CHLORIDE.** Dissolve 1 gram in 50 cc. of water and add 1 cc. nitric acid and 1 cc. of 0.1 *N* silver nitrate. Any turbidity should not be greater than is produced by 0.05 mg. of chloride in an equal volume of solution containing the quantities of reagents used in the test.

**NITRATE.** Dissolve 1 gram in 9 cc. of water containing 5 mg. sodium chloride. Add 0.7 cc. of acetic acid, 0.2 cc. of indigo carmine solution (1 to 1000), and 10 cc. of sulfuric acid. Shake thoroughly and allow to stand for 10 minutes. The blue color of the clear solution should not be completely discharged.

**Solution A.** Dissolve 5 grams in 42 cc. of water and 3 cc. acetic acid, and add 5 cc. of sulfuric acid. After standing about 10 minutes, filter the solution.

**SUBSTANCES NOT PRECIPITATED BY HYDROGEN SULFIDE.** Dilute 10 cc. of solution A to 100 cc., pass hydrogen sulfide through the solution to precipitate all the lead, and filter. Evaporate 50 cc. of the filtrate to dryness and ignite gently. The weight of the residue should not exceed 0.0015 gram.

**COPPER.** To 25 cc. of solution A add about 0.05 gram aluminum chloride and a few crystals of ammonium persulfate. Neutralize with ammonium hydroxide and add a very slight excess. Heat to boiling, cool, and filter. Save the precipitate for the determination of iron. Neutralize the filtrate to phenolphthalein, add 0.25 cc. of acetic acid in excess and 0.25 cc. of a freshly prepared 10% solution of potassium ferrocyanide. Any pink color produced should not exceed that produced by 0.125 mg. of copper in an equal volume of solution containing the quantities of reagents used in the test.

**IRON.** Wash the precipitate of iron and aluminum hydroxide obtained in the previous test sufficiently to remove most of the acetate. Dissolve the precipitate in 10 cc. of hot dilute hydrochloric acid (1 + 5), wash the paper and dilute to 50 cc. Dilute



cc. of this solution to 45 cc. and add a few crystals of ammonium persulfate, 3 cc. of hydrochloric acid, and 3 cc. of a 30% solution of ammonium thiocyanate. The color should not be more than is produced by 0.05 mg. of ferric iron in 45 cc. of water to which are added 3 cc. of hydrochloric acid and 3 cc. of a 30% solution of ammonium thiocyanate.

## Manganese Sulfate Monohydrate

Replacing previously published specification for Manganese sulfate (12).

### Requirements

INSOLUBLE MATTER. Not more than 0.010%.  
CHLORIDE (Cl). Not more than 0.005%.  
SUBSTANCES NOT PRECIPITATED BY AMMONIUM SULFIDE. Not more than 0.50%.  
IRON (Fe). Not more than 0.002%.  
HEAVY METALS (as Pb). To pass test (limit about 0.005%).  
NICKEL (Ni). Not more than 0.02%.  
ZINC (Zn). Not more than 0.01%.  
SUBSTANCES REDUCING PERMANGANATE. To pass test.

### Tests

INSOLUBLE MATTER. Dissolve 10 grams in 130 cc. of hot water, and heat on the steam bath for 1 hour. Filter through a tared filtering crucible, wash thoroughly, and dry at 105–110° C. The weight of the insoluble residue should not exceed 0.010 gram.

CHLORIDE. Dissolve 1 gram in 50 cc. of water, add 1 cc. of formic acid and 1 cc. of 0.1 N silver nitrate. Any turbidity should not exceed that produced by 0.05 mg. of chloride in an equal volume of solution containing the quantities of reagents used in the test.

SUBSTANCES NOT PRECIPITATED BY AMMONIUM SULFIDE. Dissolve 2 grams in about 90 cc. of water and add a sufficient quantity of freshly prepared solution of ammonium sulfide to precipitate manganese. Heat on the steam bath for 30 minutes, cool, dilute to 100 cc., mix thoroughly, and filter. Evaporate 50 cc. of filtrate to dryness in a tared dish, ignite gently, and weigh. The weight of the residue should not exceed 0.0050 gram.

IRON. Dissolve 1 gram in 45 cc. of water, and add 2 cc. of hydrochloric acid, a few crystals of ammonium persulfate, and 3 cc. of a 30% solution of ammonium thiocyanate. Any red color should not be more than is produced by 0.02 mg. of iron in an equal volume of solution containing the quantities of reagents used in the test.

HEAVY METALS. *Solution A.* Dissolve 1 gram in 20 cc. of water and 1 cc. of 1 N hydrochloric acid. *Solution B.* Dissolve 1 gram in 10 cc. of water and 1 cc. of 1 N hydrochloric acid and 10 cc. of hydrogen sulfide water. *Solution B* should be no darker than *Solution A*.

NICKEL. Dissolve 1 gram in 200 cc. of water. To 20 cc. of this solution add 2 grams of sodium acetate and 10 cc. of hydrogen sulfide water, and allow to stand 1 minute. Add 5 cc. of acetic acid. Any color should not be more than is produced by 0.02 mg. of nickel in 20 cc. of solution containing the quantities of reagents used in the test.

ZINC. Dissolve 2 grams in 50 cc. of water containing 2 cc. of formic acid and add, with thorough agitation, 1 cc. of a 1% solution of potassium ferrocyanide. No turbidity should be produced in 5 minutes.

SUBSTANCES REDUCING PERMANGANATE. Dissolve 7.5 grams in 100 cc. of water containing 3 cc. of sulfuric acid and 3 cc. of phosphoric acid. To this solution add 0.1 cc. of 0.1 N potassium permanganate in excess of the amount required to produce a pink color in 200 cc. of water containing 3 cc. of sulfuric acid and 3 cc. of phosphoric acid. The pink color should not be entirely discharged at the end of 1 minute.

## Mercuric Oxide, Yellow

### Requirements

INSOLUBLE IN HYDROCHLORIC ACID. Not more than 0.030%.  
NONVOLATILE MATTER. Not more than 0.050%.  
CHLORIDE (Cl). Not more than 0.025%.  
SULFATE (SO<sub>4</sub>). Not more than 0.020%.  
TOTAL NITROGEN (N). Not more than 0.005%.  
IRON (Fe). Not more than 0.003%.

### Tests

INSOLUBLE IN HYDROCHLORIC ACID. Dissolve 3 grams in 30 cc. of diluted hydrochloric acid (1 + 3) and heat on the steam

bath for 1 hour. Filter through a tared filtering crucible, wash well with water, and dry at 105–110° C. The weight of the insoluble residue should not exceed 0.0009 gram.

NONVOLATILE MATTER. Ignite 3 grams in a tared porcelain dish in a well-ventilated hood, cool, and weigh. The weight of the residue should not exceed 0.0015 gram. Save the residue for the determination of iron.

CHLORIDE. Dissolve 1 gram in 50 cc. of water and 1 cc. of formic acid. Add, dropwise, a 10% solution of sodium hydroxide until a small amount of permanent precipitate is formed. Digest under a reflux condenser until all the mercury is reduced to metal and the solution is clear. Cool, filter through a paper that has been washed free of chlorides, and dilute to 100 cc. Dilute 20 cc. of this solution to a total volume of 50 cc. containing 1 cc. of nitric acid and 1 cc. of 0.1 N silver nitrate. Any turbidity should not be more than that in an equal volume of solution containing 0.05 mg. of chloride and the quantities of reagents used in the test.

*Solution A.* Dissolve 5 grams in 50 cc. of water and 3 cc. of formic acid. Digest under a reflux condenser until all the mercury is reduced to metal and the supernatant liquid is clear. Cool, filter through a well-washed paper, and dilute to 100 cc.

SULFATE. To 10 cc. of solution A add 0.01 gram of sodium carbonate and evaporate to dryness. Dissolve the residue in 10 cc. of water and add 1 cc. of 1 N hydrochloric acid. Filter if necessary and add 1 cc. of a 10% solution of barium chloride. Any turbidity should not be more than that produced in a solution containing 0.10 mg. of sulfate, 0.6 cc. of formic acid, and 0.01 gram of sodium carbonate treated in the same way as the solution of the sample.

TOTAL NITROGEN. Dilute 10 cc. of solution A to 55 cc. in a flask suitable for an ammonia distillation, add 15 cc. of a 10% solution of sodium hydroxide and 1 gram of aluminum wire or small chips. Connect the flask, using a spray trap, to a condenser, the tip of which dips below the surface of 10 cc. of 0.1 N hydrochloric acid. Distill over 35 cc., and add 5 cc. of a 10% solution of sodium hydroxide and 2 cc. of Nessler's solution. Any color produced should not be greater than that produced in a solution containing 0.025 mg. of ammonia and 0.33 cc. of formic acid treated in the same way as the solution of the sample.

IRON. Dissolve the residue obtained in the test for non-volatile matter by warming with 9 cc. of hydrochloric acid and a few drops of nitric acid, and dilute to 150 cc. To 50 cc. of this solution add 3 cc. of a 30% solution of ammonium thiocyanate. Any red color should not be greater than that produced by 0.03 mg. of iron in an equal volume of solution containing the quantities of reagents used in the test.

## Phosphorus Pentoxide

### Requirements

ASSAY (P<sub>2</sub>O<sub>5</sub>). Not less than 97.0%.  
INSOLUBLE MATTER. Not more than 0.02%.  
PHOSPHORUS TRIOXIDE (P<sub>2</sub>O<sub>3</sub>). Not more than 0.02%.  
AMMONIA (NH<sub>3</sub>). Not more than 0.01%.  
ARSENIC (As). Not more than 0.005%.  
HEAVY METALS (as lead). To pass test (limit about 0.01%).

### Tests

ASSAY. Weigh accurately about 1.5 grams, dissolve in 100 cc. of water, evaporate to 25 cc., and dissolve 5 grams of sodium chloride in the solution. Cool the solution to 15° C. and titrate at this temperature with normal sodium hydroxide, using 3 drops of phenolphthalein indicator solution. Each cubic centimeter of normal alkali consumed corresponds to 0.03549 gram of P<sub>2</sub>O<sub>5</sub>.

INSOLUBLE MATTER. Dissolve 5 grams in 40 cc. of water (the phosphorus pentoxide must be added to the water in small quantities to prevent excessive heating and sputtering) and warm if necessary to complete solution. Filter through a tared filtering crucible, and set aside the filtrate for solution A. Wash the residue well with water and dry at 105–110° C. The weight of the insoluble residue should not exceed 0.0010 gram.

*Solution A.* Make up the filtrate from the test for insoluble matter to 50 cc.

PHOSPHORUS TRIOXIDE. To 30 cc. of solution A add 0.20 cc. of 0.1 N potassium permanganate solution. Heat to boiling and allow to digest on the steam bath for 10 minutes. The pink color should not be entirely discharged.

AMMONIA. Dilute 5 cc. of solution A to 40 cc., add 10 cc. of a 10% solution of sodium hydroxide and 2 cc. of Nessler's solution. The color should not be more than is produced by 0.05 mg. of ammonia in an equal volume containing the quantities of reagents used in the test.

ARSENIC. Determine the arsenic by the Gutzeit procedure in 1 cc. of solution A. The stain should not be greater than is produced by 0.005 mg. of arsenic.



**HEAVY METALS.** Dilute 5 cc. of solution A to 10 cc. and exactly neutralize with ammonium hydroxide, using 3 drops of phenolphthalein solution as the indicator. Add 50 cc. of 1 *N* sulfuric acid, 5 cc. of hydrogen sulfide water, and dilute to 100 cc. Any brown color immediately observed should not be more than is produced by 0.05 mg. of lead in an equal volume of water containing 5 cc. of hydrogen sulfide water.

### Sodium Phosphate, Dibasic, Heptahydrate ( $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$ )

Replacing specification for Sodium Phosphate, Dibasic (Disodium Hydrogen Phosphate), previously published (4).

#### Requirements

**INSOLUBLE MATTER.** Not more than 0.005%.  
**WATER OF CRYSTALLIZATION.** 43 to 50%.  
**NEUTRALITY.** To pass test.  
**CHLORIDE (Cl).** Not more than 0.001%.  
**NITROGEN COMPOUNDS (as N).** Not more than 0.001%.  
**SULFATE ( $\text{SO}_4$ ).** Not more than 0.010%.  
**ARSENIC (As).** Not more than 0.0005%.  
**HEAVY METALS.** To pass test (limit about 0.001% as lead).  
**IRON (Fe).** Not more than 0.001%.

#### Tests

**INSOLUBLE MATTER.** Dissolve 10 grams in 100 cc. of water, allow to stand on the steam bath for 1 hour, filter through a tared filtering crucible, wash well with water, dry at 105–110° C., cool, and weigh. The weight of the residue should not exceed 0.0005 gram.

**WATER OF CRYSTALLIZATION.** Weigh accurately about 1 gram. Dry to constant weight at 105–110° C. The loss in weight should be from 43 to 50%.

**NEUTRALITY.** Dissolve 3 grams in 30 cc. of water at 15° C. and add 3 drops of phenolphthalein solution. A red color should be produced which should be discharged by the addition of 0.8 cc. of *N* hydrochloric acid. Boil the solution for 2 minutes, cool to 15° C., and dilute to the original volume with cold water. The resulting solution should show no pink color.

**CHLORIDE.** Dissolve 2 grams in 20 cc. of water, and add 3 cc. of nitric acid and 1 cc. of 0.1 *N* silver nitrate. Any turbidity should not be greater than is produced by 0.02 mg. of chloride in an equal volume of solution containing the quantities of reagent used in the test.

**NITROGEN COMPOUNDS.** Dissolve 2 grams in 30 cc. of water, add 20 cc. of a 10% solution of sodium hydroxide and 0.5 gram of aluminum wire in small pieces, and allow to stand for 3 hours protected from loss or access of ammonia. Decant 25 cc. and add 2 cc. of Nessler's solution. The color should not be more than that produced in a similar aliquot of a complete blank to which has been added 0.02 mg. of nitrogen.

**SULFATE.** Dissolve 10 grams in 100 cc. of water, add 7 cc. of hydrochloric acid, and heat to boiling. Add 5 cc. of a 10% solution of barium chloride, heat on the steam bath for 2 hours, and allow to stand overnight. If a precipitate forms, filter, wash, ignite, and weigh. The weight of the ignited precipitate should not be more than 0.0025 gram in excess of the weight obtained from a complete blank on the reagents used, including filtration.

**ARSENIC.** Determine the arsenic in a 2-gram sample by the modified Gutzeit method. The stain should not be more than is produced by 0.010 mg. of arsenic.

**HEAVY METALS.** Dissolve 5 grams in 40 cc. of water and exactly neutralize the solution with 1 *N* sulfuric acid, using 3 drops of 1% phenolphthalein solution as indicator. Add 15 cc. of 1 *N* sulfuric acid and 5 cc. of hydrogen sulfide water, and dilute to 100 cc. Any brown color which is immediately developed should not be greater than that produced by 0.05 mg. of lead in an equal volume of an aqueous solution containing 5 cc. of hydrogen sulfide water.

**IRON.** Dissolve 5 grams in 100 cc. of water. Dilute 20 cc. of this solution to 40 cc., add 1 cc. of ammonium hydroxide, and 5 cc. of hydrogen sulfide water. Any green color should not be greater than is produced by 0.01 mg. of iron in an equal volume of solution containing the quantities of reagents used in the test.

### Zinc

Replacing previously published specifications for Zinc, Low in Arsenic, and Zinc, Low in Lead and Iron (6).

#### Requirements

**ARSENIC (As).** Not more than 0.00002%.  
**IRON (Fe).** Not more than 0.01%.  
**LEAD (Pb).** Not more than 0.01%.

#### Tests

**ARSENIC.** Test 10 grams by the modified Gutzeit method, using 12 cc. of sulfuric acid or 20 cc. of hydrochloric acid, diluted with about 70 cc. of water. Any stain produced should not be more than is produced by 0.002 mg. of arsenic, allowance being made for the blank.

**Solution A.** Dissolve 2 grams in 15 cc. of diluted hydrochloric acid (1 + 1). When solution is nearly complete add 1 cc. nitric acid and heat to boiling, or until any residue from the zinc is dissolved. Cool and dilute to 100 cc.

**IRON.** To 25 cc. of solution A add 2 cc. of hydrochloric acid and 3 cc. of a 30% solution of ammonium thiocyanate, and dilute to 50 cc. Any red color should not be more than is produced by 0.05 mg. of iron in an equal volume containing the quantities of reagents used in the test.

**LEAD.** Dilute 5 cc. of solution A to 20 cc. and add ammonium hydroxide until a small amount of permanent precipitate is formed. Carefully add nitric acid in sufficient amount just to dissolve the precipitate. Pour the resulting solution into 50 cc. of a 10% solution of sodium cyanide, mix thoroughly, and add 0.20 cc. of a 10% solution of sodium sulfide. Any brown color should not be more than is produced by 0.01 mg. of lead in an equal volume containing the quantities of reagents used in the test.

## Corrections for Published Specifications

### Acetone (2)

#### Requirements

**SPECIFIC GRAVITY AT 25°/25° C.** Not above 0.788.

**METHANOL.** To pass test (limit about 0.1%).

#### Test

**METHANOL.** Dilute 5 cc. with water to 100 cc. To 5 cc. of the solution add 0.5 cc. of phosphoric acid and 2 cc. of a 3% solution of potassium permanganate and allow to stand for 10 minutes. Add 1.5 cc. of a 10% solution of oxalic acid and allow to stand until the solution is colorless. Add 5 cc. of diluted sulfuric acid (1 + 3) and 5 cc. of fuchsin-sulfurous acid solution. No blue-violet color should be produced in 10 minutes. To prepare the fuchsin-sulfurous acid solution, dissolve 0.2 gram of fuchsin in 120 cc. of hot water, cool, add 20 cc. of a 10% solution of sodium sulfite and 2 cc. of hydrochloric acid. Dilute the solution to 200 cc. and allow to stand until it is nearly colorless.

### Acid, Citric (5)

#### Test

**HEAVY METALS.** In the second sentence change 7 cc. of ammonium hydroxide to 10 cc. of ammonium hydroxide.

### Acid, Oxalic (1)

#### Requirements

**HEAVY METALS.** To pass test (limit about 0.001% as lead).  
**IRON (Fe).** Not more than 0.0005%.

#### Tests

**NONVOLATILE.** Ignite 5 grams in a porcelain crucible at low red heat to constant weight. The weight of the residue should not exceed 0.0010 gram. Save the residue.

**HEAVY METALS.** To the residue from the test for nonvolatile matter add about 0.5 cc. of hydrochloric acid and 0.1 cc. of nitric acid, and evaporate to dryness. Dissolve the residue in 1 cc. of 0.1 *N* hydrochloric acid and dilute to 10 cc. Dilute 5 cc. of the solution to 40 cc. and add 10 cc. of hydrogen sulfide water. Any brown color should not be greater than is produced by 0.025 mg. of lead in an equal volume of solution containing the quantities of reagents used in the test.

**IRON.** Dilute the remaining 5 cc. of the solution of the nonvolatile residue to 45 cc., and add 2 cc. of hydrochloric acid, a few crystals of ammonium persulfate, and 3 cc. of a 30% solution of ammonium thiocyanate. Any red color should not be greater than is produced by 0.0125 mg. of ferric iron in an equal volume of solution containing the quantities of reagents used in the test.



**Acid, Phosphoric (4)****Test**

NITRATE. Dilute 2 cc. to 10 cc. containing 5 mg. of sodium fluoride. Add 0.10 cc. of indigo carmine solution (1 to 1000) and 0.5 cc. of sulfuric acid. The blue color should not be completely discharged in 5 minutes.

**Aluminum and Potassium Sulfate (2)****Requirement**

SODIUM (Na). To pass test (limit about 0.02%).

**Test**

SODIUM. A 10% solution in hot water tested with a platinum wire in a colorless flame should impart no pronounced yellow color to the flame.

**Ammonium Oxalate (1)****Requirements**

HEAVY METALS. To pass test (limit about 0.001% as lead). IRON (Fe). Not more than 0.0005%.

**Tests**

NONVOLATILE. Ignite 5 grams in a porcelain crucible at a low heat to constant weight. The weight of the residue should not exceed 0.0010 gram. Save the residue.

HEAVY METALS. To the residue from the test for nonvolatile matter add about 0.5 cc. of hydrochloric acid and 0.1 cc. of nitric acid and evaporate to dryness. Dissolve the residue in 1 cc. of 10% hydrochloric acid and dilute to 10 cc. Dilute 5 cc. of this solution to 40 cc. and add 10 cc. of hydrogen sulfide water. Any color should not be greater than is produced by 0.025 mg. of iron in an equal volume of solution containing the quantities of reagents used in the test.

IRON. Dilute the remaining 5 cc. of the solution of the nonvolatile residue to 45 cc., and add 2 cc. of hydrochloric acid, a few drops of ammonium persulfate, and 3 cc. of a 30% solution of ammonium thiocyanate. Any red color should not be greater than is produced by 0.0125 mg. of ferric iron in an equal volume of solution containing the quantities of reagents used in the test.

**Ammonium Persulfate (2)****Requirement**

CHLORIDE AND CHLORATE (as Cl). Not more than 0.001%.

**Test**

CHLORIDE AND CHLORATE. Mix 2 grams with 2 grams of sodium carbonate and heat until no more gas is evolved. Dissolve the residue in 20 cc. of water, neutralize with nitric acid, add an excess of 1 cc., and add 1 cc. of a 0.1 N silver nitrate solution. Any turbidity should not be greater than that produced by 0.02 mg. of chloride in an equal volume of solution containing the quantities of reagents used in the test.

**Barium Carbonate (3)****Tests**

NITRATE. To 3 grams in 23 cc. of water containing 15 mg. of sodium chloride add 7 cc. of acetic acid. To 10 cc. of this solution add 0.20 cc. of indigo carmine (1 to 1000) and 10 cc. of sulfuric acid. Heat on the steam bath for 1 hour and stir the precipitate thoroughly several times. The blue color of the clear solution should not be completely discharged.

IRON AND CALCIUM SALTS. Dissolve 3 grams in 30 cc. of water and 4 to 5 cc. of hydrochloric acid, and evaporate to dryness. Powder the residue, add 30 cc. of absolute alcohol, and allow to stand for 30 minutes with occasional shaking. Filter and evaporate 20 cc. of the filtrate to a few cubic centimeters, add about 1 cc. of diluted sulfuric acid (1 + 9), evaporate to dryness, ignite, and weigh. The weight of the residue should not exceed 0.0060 gram.

**Barium Chloride (1)****Test**

NITRATE AND CHLORATE. Dissolve 1 gram in 10 cc. of water, add 0.20 cc. of indigo carmine solution (1 to 1000) and 10 cc.

of sulfuric acid. Stir constantly during the addition of the sulfuric acid. Heat on the steam bath for 1 hour and stir the precipitate thoroughly several times. The blue color of the clear solution should not be completely discharged.

**Calcium Carbonate (7)****Tests**

SULFATE. The weight of the ignited precipitate should not exceed 0.0024 gram.

MAGNESIUM AND ALKALI SALTS. The fourth sentence should read, "To 125 cc. of the filtrate add 0.5 cc. of sulfuric acid, evaporate to dryness, ignite at 700-750° C. for 30 minutes, and weigh."

**Calcium Carbonate, Low in Alkalies (7)****Tests**

SULFATE. The weight of the ignited precipitate should not exceed 0.0024 gram.

MAGNESIUM AND ALKALI SALTS. The fourth sentence should read, "To 125 cc. of the filtrate add 0.5 cc. of sulfuric acid, evaporate to dryness, ignite at 700-750° C. for 30 minutes, and weigh."

**Calcium Chloride (CaCl<sub>2</sub>·2H<sub>2</sub>O) (7)****Tests**

NITRATE. Use 0.10 cc. of indigo carmine solution (1 to 1000).

MAGNESIUM AND ALKALI SALTS. The fourth sentence should read, "To 125 cc. of the filtrate add 0.5 cc. of sulfuric acid, evaporate to dryness, ignite at 700-750° C. for 30 minutes, and weigh."

**Calcium Chloride, Anhydrous (5)****Test**

MAGNESIUM AND ALKALI SALTS. The second sentence should read, "To 100 cc. of the filtrate add 0.5 cc. of sulfuric acid, evaporate to dryness, and ignite at 700-750° C. for 30 minutes."

**Carbon Disulfide (4)**

NOTE. Carbon disulfide should be supplied and stored in amber glass containers and protected from direct sunlight.

**Chloroform (12)**

NOTE. This reagent normally contains about 0.75% of alcohol as a stabilizer.

**Cupric Oxide, Powdered (6)****Test**

CARBON COMPOUNDS. Ignite 0.6 gram in a stream of carbon dioxide-free air and pass the evolved gases into 20 cc. of diluted ammonium hydroxide (1 + 7), add 2 cc. of a 10% solution of barium chloride, and compare immediately with the standard. The turbidity should not be greater than that produced by 2 cc. of 0.01 N sodium carbonate in an equal volume containing the quantities of reagents used in the test.

**Cuprous Chloride (7)****Requirement**

ARSENIC (As). Not more than 0.002%.

**Ether (6)**

NOTE. Ether conforming to this specification normally contains about 2% of alcohol and about 0.5% of water.

**Requirements**

SPECIFIC GRAVITY 25°/25° C. 0.712 to 0.714.

FOREIGN ODOR. To pass test.

**Test**

FOREIGN ODOR. Allow 10 cc. to evaporate spontaneously to a volume of about 1 cc. in a dry evaporating dish: no foreign odor



should be perceptible. Transfer this residue to a piece of clean, odorless absorbent paper: no foreign odor should be perceptible when the last traces of ether evaporate from the paper.

### Ether, Absolute (6)

#### Requirement

FOREIGN ODOR. To pass test.

#### Test

FOREIGN ODOR. Allow 10 cc. to evaporate spontaneously to a volume of about 1 cc. in a dry evaporating dish: no foreign odor should be perceptible. Transfer this residue to a piece of clean, odorless absorbent paper: no foreign odor should be perceptible when the last traces of ether evaporate from the paper.

### Ferric Ammonium Sulfate (2)

#### Requirement

NITRATE ( $\text{NO}_3$ ). To pass test (limit about 0.01%).

#### Test

NITRATE. Dissolve 2 grams in 15 cc. of water, heat, and pour the hot solution into 6 cc. of diluted ammonium hydroxide (1 + 1). Filter and allow to drain well; to the filtrate add 0.10 cc. of indigo carmine solution (1 to 1000) and 15 cc. of sulfuric acid. The blue color should not be completely discharged in 5 minutes.

### Ferric Chloride (9)

#### Requirement

SULFATE ( $\text{SO}_4$ ). Not more than 0.01%.

#### Tests

NITRATE. To 15 cc. of solution A, add 0.10 cc. of indigo carmine solution (1 to 1000), and 15 cc. of sulfuric acid. The blue color should not be completely discharged in 5 minutes.

SULFATE. Concentrate 120 cc. of solution A to 75 cc., add 1 cc. of hydrochloric acid, heat to boiling, add 10 cc. of a 10% solution of barium chloride, and allow to stand overnight. Filter, wash thoroughly, ignite, and weigh. The weight of the ignited precipitate should not exceed the weight obtained from a complete blank by more than 0.0010 gram.

### Glycerol (12)

#### Requirement

ASSAY. Not less than 95% by volume.

Use above title in place of "Specific Gravity at 25°/25° C."

#### Test

ASSAY. The specific gravity at 25°/25° C. should not be less than 1.249.

### Hydrogen Peroxide (11)

NOTE. This reagent should be stored in ceresin or ceresin-lined containers.

### Lead Acetate (7)

#### Tests

*Solution A.* Dissolve 5 grams in 42 cc. of water and 3 cc. of acetic acid, and add 5 cc. of sulfuric acid. After standing for about 10 minutes, filter the solution.

COPPER. To 25 cc. of solution A add about 0.05 gram of aluminum chloride and a few crystals of ammonium persulfate. Neutralize with ammonium hydroxide and add a very slight excess. Heat to boiling, cool, and filter. Save the precipitate for the determination of iron. Neutralize the filtrate to phenolphthalein. Add 0.25 cc. of acetic acid and 0.25 cc. of a freshly prepared 10% solution of potassium ferrocyanide. Any pink color should not exceed that produced by 0.125 mg. of copper in an equal volume of solution containing the quantities of reagents used in the test.

IRON. Wash the precipitate of iron and aluminum hydroxides obtained in the previous test sufficiently to remove most of the acetate. Dissolve the precipitate in 10 cc. of hot dilute hydro-

chloric acid (1 + 5), wash the paper, and dilute to 45 cc. Add a few crystals of ammonium persulfate, 3 cc. of hydrochloric acid and 3 cc. of a 30% solution of ammonium thiocyanate. The color should not be more than is produced by 0.025 mg. of iron in an equal volume of solution containing the quantities of reagents used in the test.

SUBSTANCES NOT PRECIPITATED BY HYDROGEN SULFIDE. Dilute 20 cc. of solution A to 100 cc., pass hydrogen sulfide through the solution to precipitate all the lead, and filter. Evaporate 50 cc. of the filtrate to dryness and ignite gently. The weight of the residue should not exceed 0.0005 gram.

### Lead Dioxide (3)

#### Test

OTHER HYDROGEN SULFIDE METALS. Dissolve 0.5 gram in 10 cc. of nitric acid and 10 cc. of 3% hydrogen peroxide solution. Add 5 cc. of sulfuric acid and evaporate to fumes. Cautiously dilute to 40 cc., allow to stand until the solution is cool and the precipitate has settled. Filter, do not wash, add 10 cc. of sulfuric acid, and boil until the odor of sulfur dioxide disappears. Cool, dilute to 50 cc., and pass hydrogen sulfide through the solution. No red color should be produced, and any darkening should not be more than is produced by 0.10 mg. of lead in an equal volume of solution containing the quantities of reagents used in the test.

### Potassium and Sodium Tartrate (6)

#### Requirement

AMMONIA ( $\text{NH}_3$ ). Not more than 0.005%.

#### Test

AMMONIA. Dissolve 1 gram in 45 cc. of water, add 2 cc. of 10% solution of sodium hydroxide and 2 cc. of Nessler's solution. The color should not be more than is produced by 0.05 mg. of ammonia in an equal volume containing the quantities of reagents used in the test.

### Potassium Bisulfate, Fused (9)

#### Requirements

HEAVY METALS. To pass test (limit about 0.001% as lead).  
IRON (Fe). Not more than 0.002%.

#### Tests

*Solution A.* Dissolve 5 grams in 45 cc. of water, add 5 cc. of hydrochloric acid, and boil gently for 10 minutes. Cool and restore volume to 50 cc.

HEAVY METALS. Neutralize 20 cc. of solution A to phenolphthalein with ammonium hydroxide, add 0.5 cc. of acetic acid and 10 cc. of hydrogen sulfide water. The color produced should not be more than that produced by 0.02 mg. of lead and 2 cc. of hydrochloric acid in 20 cc. of solution treated in the same way as the solution of the sample.

IRON. Dilute 10 cc. of solution A to 50 cc., add a few crystals of ammonium persulfate and 3 cc. of a 30% solution of ammonium thiocyanate. The color produced should not be more than that produced by 0.02 mg. of iron and 1 cc. of hydrochloric acid in an equal volume of solution containing the quantities of reagents used in the test.

### Potassium Chlorate (3)

#### Test

NITROGEN. Change the last sentence to, "The color should not be more than that produced in a similar aliquot of a complete blank to which has been added 0.02 mg. of nitrogen."

### Potassium Hydroxide (1)

#### Tests

POTASSIUM HYDROXIDE AND CARBONATE. Accurately weigh 35 to 40 grams, dissolve, and dilute to 1 liter, using carbon dioxide-free water. Use a 50-cc. aliquot of this solution for titration instead of 10 cc. of solution A.

PHOSPHATE. Neutralize 10 cc. of solution A with nitric acid and dilute to 50 cc. Add 10 cc. of nitric acid, neutralize with ammonium hydroxide using phenolphthalein indicator, and add 0.5 cc. of nitric acid in excess. Add 50 cc. of ammonium molybdate solution, shake the solution (at about 40° C.) for 5 minutes.



allow to stand for 0.5 hour. Any precipitate formed should be more than is formed when 0.10 mg. of phosphate is treated by the above procedure, except that the control should include residue from evaporation of the quantity of nitric acid used to neutralize 10 cc. of solution A.

**IRON.** Neutralize 25 cc. of solution A to phenolphthalein with hydrochloric acid, add 1 cc. in excess, and dilute to 50 cc. Add a few crystals of ammonium persulfate and 3 cc. of a 30% solution of ammonium thiocyanate. The red color should not exceed that produced by 0.10 mg. of iron in an equal volume of solution containing the quantities of reagents used in the test. The control should contain residue from evaporation of the quantity of hydrochloric acid used to neutralize 25 cc. of solution A.

**HEAVY METALS.** Neutralize 25 cc. of solution A to phenolphthalein with hydrochloric acid and add a slight excess. Exactly neutralize with ammonium hydroxide, add 0.5 cc. of acetic acid, and saturate with hydrogen sulfide. The darkening should not exceed that produced by 0.15 mg. of silver in an equal volume of solution containing the quantities of reagents used in the test. The standard should contain residue from evaporation of the quantity of hydrochloric acid used to neutralize 25 cc. of solution A.

## Potassium Oxalate (11)

### Requirement

**IRON (Fe).** Not more than 0.001%.

### Tests

**Solution A.** To 5 grams in a 150-cc. beaker add 5 cc. of sulfuric acid. Heat under a well-ventilated hood until decomposition is complete and heavy fumes of sulfuric acid are given off. Cool and dilute to 100 cc.

**HEAVY METALS.** Neutralize 10 cc. of solution A with ammonium hydroxide to the end point of phenolphthalein, add 0.5 cc. of acetic acid, and dilute to 25 cc. Cool and add 10 cc. of hydrogen sulfide water. The color should not be more than that produced in a solution of the same volume containing 0.5 cc. of sulfuric acid and 0.01 mg. of lead neutralized with ammonium hydroxide and otherwise treated in the same way.

**IRON.** To 20 cc. of solution A add a few crystals of ammonium persulfate and 3 cc. of a 30% solution of ammonium thiocyanate, dilute to 50 cc. The color should not be more than that produced by 0.01 mg. of iron and 1 cc. of sulfuric acid in an equal volume of solution containing the quantities of reagents used in the test.

## Sodium Bisulfate, Fused ( $\text{NaHSO}_4$ ) (5)

### Requirements

**HEAVY METALS.** To pass test (limit about 0.001% as lead). **IRON (Fe).** Not more than 0.002%.

### Tests

**Solution A.** Dissolve 5 grams in 45 cc. of water, add 5 cc. of hydrochloric acid, and boil gently for 10 minutes. Cool and dilute to 50 cc.

**HEAVY METALS.** Neutralize 20 cc. of solution A to phenolphthalein with ammonium hydroxide, add 0.5 cc. of acetic acid and 10 cc. of hydrogen sulfide water. The color produced should not be more than that produced by 0.02 mg. of lead and 2 cc. of hydrochloric acid in 20 cc. of solution treated in the same way as solution of the sample.

**IRON.** Dilute 10 cc. of solution A to 50 cc., add a few crystals of ammonium persulfate and 3 cc. of a 30% solution of ammonium thiocyanate. The color produced should not be more than that produced by 0.02 mg. of iron and 1 cc. of hydrochloric acid in an equal volume of solution containing the quantities of reagents used in the test.

## Sodium Carbonate, Anhydrous (2)

### Test

**SILVER NITRATE.** The first sentence should read, "Dissolve 1 gram in 10 cc. of warm water, add 2 cc. of nitric acid, cool, and add 1 cc. of 1N silver nitrate solution."

## Sodium Hydroxide (1)

### Tests

**SODIUM HYDROXIDE AND CARBONATE.** Accurately weigh 35 grams, dissolve, and dilute to 1 liter, using carbon dioxide-

free water. Use a 50-cc. aliquot of this solution for the titration instead of 10 cc. of solution A.

**PHOSPHATE.** Neutralize 10 cc. of solution A with nitric acid and dilute to 50 cc. Add 10 cc. of nitric acid, neutralize with ammonium hydroxide, using phenolphthalein indicator, and add 0.5 cc. of nitric acid in excess. Add 50 cc. of ammonium molybdate solution, shake the solution (at about 40° C.) for 5 minutes, and allow to stand for 0.5 hour. Any precipitate formed should not be more than is formed when 0.10 mg. of phosphate is treated by the above procedure, except that the control should include the residue from evaporation of the quantity of nitric acid used to neutralize 10 cc. of solution A.

**IRON.** Neutralize 25 cc. of solution A to phenolphthalein with hydrochloric acid, add 1 cc. in excess, and dilute to 50 cc. Add a few crystals of ammonium persulfate and 3 cc. of a 30% solution of ammonium thiocyanate. The red color should not exceed that produced by 0.10 mg. of iron in an equal volume of solution containing the quantities of reagents used in the test. The standard should contain residue from evaporation of the quantity of hydrochloric acid used to neutralize 25 cc. of solution A.

**HEAVY METALS.** Neutralize 25 cc. of solution A to phenolphthalein with hydrochloric acid and add a slight excess. Exactly neutralize with ammonium hydroxide, add 0.5 cc. of acetic acid, and saturate with hydrogen sulfide. The darkening should not exceed that produced by 0.15 mg. of silver in an equal volume of solution containing the quantities of reagents used in the test. The standard should contain the residue from evaporation of the quantity of hydrochloric acid used to neutralize 25 cc. of solution A.

## Sodium Nitrate (4)

### Requirement

**CALCIUM, MAGNESIUM, AND AMMONIUM HYDROXIDE PRECIPITATE.** Use this title instead of present title of "Calcium and Magnesium Precipitate". The limit remains the same.

### Test

**CALCIUM, MAGNESIUM, AND AMMONIUM HYDROXIDE PRECIPITATE.** The second sentence should read, "If any precipitate is formed, filter, wash with diluted ammonium hydroxide (1 + 9), ignite, and weigh."

## Sodium Oxalate (1)

### Requirements

**NEUTRALITY.** To pass test (limit of alkalinity equivalent to 0.042%  $\text{Na}_2\text{CO}_3$ , limit of acidity equivalent to 0.022% of  $\text{NaHC}_2\text{O}_4$ ).

**HEAVY METALS.** To pass test (limit about 0.002% as lead). **IRON (Fe).** Not more than 0.001%.

### Tests

**Solution A.** To 5 grams in a 150-cc. beaker add 5 cc. of sulfuric acid. Heat under a well-ventilated hood until decomposition is complete and heavy fumes of sulfuric acid are given off. Cool and dilute to 100 cc.

**HEAVY METALS.** Neutralize 10 cc. of solution A with ammonium hydroxide to the end point of phenolphthalein, add 0.5 cc. of acetic acid, and dilute to 25 cc. Cool and add 10 cc. of hydrogen sulfide water. The color should not be more than that produced in a solution of the same volume containing 0.5 cc. of sulfuric acid and 0.01 mg. of lead neutralized with ammonium hydroxide and otherwise treated in the same way.

**IRON.** To 20 cc. of solution A add a few crystals of ammonium persulfate and 3 cc. of a 30% solution of ammonium thiocyanate, and dilute to 50 cc. The color should not be more than that produced by 0.01 mg. of iron and 1 cc. of sulfuric acid in an equal volume of solution containing the quantities of reagents used in the test.

## Sodium Peroxide (4)

### Test

**IRON.** Dissolve 1 gram in 10 cc. of water, add 5 cc. of hydrochloric acid, and evaporate to dryness. Treat the residue with 3 cc. of hydrochloric acid, dissolve in 45 cc. of water, add a few crystals of ammonium persulfate, and 3 cc. of a 30% solution of ammonium thiocyanate. The color should not be greater than that produced by 0.03 mg. of ferric iron in an equal volume of solution containing the quantities of reagents used in the test.



**Sodium Sulfate, Anhydrous (2)****Test**

CALCIUM, MAGNESIUM, AND AMMONIUM HYDROXIDE PRECIPITATE. Second sentence should read, "Allow to stand overnight."

**Sodium Sulfite, Anhydrous (11)****Requirements**

ASSAY. Not less than 97%  $\text{Na}_2\text{SO}_3$ .

FREE ALKALI. To pass test (limit of alkalinity equivalent to 0.15%  $\text{Na}_2\text{CO}_3$ ).

**Test**

FREE ALKALI. The last sentence should read, "Not more than 0.3 cc. of 0.1 *N* acid should be required to neutralize the solution."

**Sodium Tungstate (12)****Test**

ALKALINITY. Dissolve 2 grams in 50 cc. of cold water and add 2 drops of thymol blue indicator. A blue color should be produced which is changed to a yellow color by the addition of more than 0.4 cc. of 0.1 *N* acid.

**Specifications Previously Published**

- (1) Committee on Analytical Reagents, *IND. ENG. CHEM.*, 17, (1925).
- (2) *Ibid.*, 18, 636, 759 (1926).
- (3) *Ibid.*, 19, 645 (1927).
- (4) *Ibid.*, 19, 1369 (1927).
- (5) *Ibid.*, 20, 979 (1928).
- (6) *IND. ENG. CHEM., ANAL. ED.*, 1, 171 (1929).
- (7) *Ibid.*, 2, 351 (1930).
- (8) *Ibid.*, 3, 221 (1931).
- (9) *Ibid.*, 4, 154 (1932).
- (10) *Ibid.*, 4, 347 (1932).
- (11) *Ibid.*, 5, 289 (1933).
- (12) *Ibid.*, 12, 631 (1940).

PRESENTED in connection with the report of the Committee on Analytical Reagents at the 105th Meeting of the AMERICAN CHEMICAL SOCIETY, Detroit, Mich.

# Destructive Irradiation Technique of Spectrophotometric Vitamin A Assay

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DURING the past 15 years many studies have been made of the two in vitro methods of vitamin A assay in an attempt to replace the laborious and time-consuming animal-feeding method by a more rapid and precise means of determining vitamin A potency.

The spectrophotometric assay, while rapid, finds simple application limited to fish liver oils and food oils of fairly high potency. As the potency of the oil decreases, the extraneous absorption at the wave length of the maximum absorption of vitamin A becomes greater, and there is therefore a lower limit of potency, below which the spectrophotometric technique gives rise to apparent potencies greater than the actual vitamin A content. The practice of saponifying oils of low potency lowers the limit of applicability somewhat.

The colorimetric method of Carr and Price (2) has been used to some extent in miscellaneous products containing vitamin A, particularly to trace the occurrence and storage in the various tissues of the animal body (1, 3, 4, 13).

In 1934 the International Committee approved the spectrographic method as an alternate to the biological assay (9) but it has, as yet, been used almost exclusively in the assay of fish liver oils. The method has been shown to be applicable to butterfat (8, 14), and attempts have been made to apply it to other food extracts (5, 7).

The difficulty in obtaining an extract of a food product or animal tissue, which would be sufficiently free from interfering extraneous absorption to measure the vitamin absorption directly, suggests the use of the destructive irradiation technique as first suggested by Peacock (15). As was pointed out by Demarest (6), the validity of this method depends upon three assumptions: (1) the irradiation process destroys vitamin A, the end product of the destruction having no appreciable absorption at 3280 Å. (2) Substances present other than vitamin A undergo no appreciable change in their absorption at 3280 Å. during the irradiation. (3) The irradiation causes quantitative destruction of the vitamin present. Whether or not these three conditions can be satisfied seems still to be a subject of controversy (6, 12).

Since absorption of ultraviolet radiation is characteristic of unsaturated organic linkages and of conjugated systems in particular, it was felt that filtered radiations from a mercury vapor arc, limited as closely as possible to the absorption range of vitamin, offered the most logical opportunity of attaining specificity in the photolysis process.

**APPARATUS**

The absorption was carried out by means of a Hilger medium pressure quartz spectrograph combined with a double disk rotating sector photometer, the source of radiation being a water-cooled, low pressure, hydrogen discharge tube. The spectrograph slit was kept at a constant setting of approximately 0.09 mm.

The spectrograms were made using fused quartz absorption cells, similar to Hilger type C, and were recorded on 10 × 25 cm. (4 × 10 inch) photographic plates (Eastman 33), isodens points being picked out by visual comparison with the aid of an opal-glass spectrum viewer and a small hand lens (4×).

Destructive irradiations were carried out with a Hano quartz mercury vapor arc, laboratory model, 110 volt, direct current, the lamp always being turned on 10 minutes before radiations were begun. The spectrograph cell containing the solution to be irradiated was placed in a small, water-jacketed can (5 cm. deep, 5 cm. in diameter) which was fitted to be covered with the filter or filters desired. [Two filters were used: (1) a purple Corex A (C. G. No. 986), 3 mm. thick and 5 cm. square (2) an optical quartz cell, 5 cm. in diameter, containing 5 cc. 0.2 *M* potassium hydrogen phthalate resulting in a depth of solution of about 1 mm.] The can was raised into the hood of the lamp during the irradiation, so that the cell being irradiated was about 18 cm. from the mid-section of the arc.

**MATERIALS**

CYCLOHEXANE. A special grade was obtained (Eastman 702) and redistilled before use. In cases where the cyclohexane contained benzene or other impurities, it was purified by treatment with fuming sulfuric acid. Each new or purified lot of solvent was compared spectrographically with distilled water and with the last solvent used.

ETHYL ALCOHOL. Ethanol 95% was treated with silver nitrate and potassium hydroxide and the filtered solvent then refluxed with *m*-phenylenediamine dihydrochloride and distilled.

WET ETHER FOR EXTRACTIONS. Anhydrous ether was shaken periodically with about 1/3 its volume of 5% aqueous

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um hydroxide for 2 to 3 hours and distilled slowly from the line solution. This solvent was always purified less than 24 hours before use.

RY ETHER. Anhydrous ether was distilled onto sodium wire used as required from the storage bottle.

## PROCEDURES

USAPONIFIABLE FRACTION OF REFERENCE OIL. The procedure of Wilkie (17) was used with the following modifications. Portion of 300 to 400 mg. of oil was taken for a sample and red for 10 minutes with 5% alcoholic potassium hydroxide. Following the ether extractions 100 cc. of water were poured through the combined ether extracts without agitation. The ether layer was drawn off and the ether solution washed with 3 to 5 cc. of water, with vigorous shaking. The resulting emulsion was broken by a final wash with 100 cc. of water, the water again poured in without agitation. The washed ether extract was filtered by suction through a layer of anhydrous sodium sulfate on a sintered-glass funnel into a ground-glass vacuum distillation apparatus. The filter and anhydrous sodium sulfate were washed with two small portions of anhydrous ether and the solvent was removed at room temperature, under reduced pressure and in an atmosphere of nitrogen. The cyclohexane was removed by means of a small, side-arm separatory funnel beneath the vacuum was broken. The cyclohexane solution was transferred to a volumetric flask and made up to volume. The remaining air was displaced with nitrogen and the spectroscopic assay run within 24 hours.

As was pointed out by McFarlane and Sutherland (12), vaseline should not be employed as a stopcock lubricant in the separatory funnels. Glycerol was used in this work and found to be satisfactory.

USAPONIFIABLE EXTRACT OF ANIMAL TISSUES. The animal tissues were dissected out, placed in a weighed cork-stoppered Erlenmeyer flask, reweighed, and covered with 5% aqueous potassium hydroxide. (Care was taken to remove all fatty tissue and the tissues were blotted carefully with paper toweling to remove excess blood.) The air in the flask was displaced with nitrogen and the tissues were stored in the dark, under refrigeration until assayed.

On removal from storage, the flasks were placed in a water bath at 60° C. for 5 minutes or until complete dissolution occurred, and the tissue solution was transferred to a 50-cc. separatory funnel. The solution was shaken with 5 cc. of ethanol followed by 25 cc. of wet ether. [As was found by Davies (3), a preliminary shaking with alcohol makes it possible to extract 90% of the vitamin A with a single ether extraction.] The ether layer was drained into a second separatory funnel and the residue extracted with 25 cc. of wet ether. The ether extracts were then combined in a reflux flask with a boiling chip and the solvent was boiled off in a 60° C. water bath.

The residue was cooled, 25 cc. of ethanol and 3 cc. of 50% aqueous potassium hydroxide were added, and the mixture was stirred in a water bath for 10 minutes, using a ground-glass re- This solution was cooled, 30 cc. of water were added, and the whole was transferred to a 250-cc. separatory funnel. From this solution the sample was treated as given above for the unsaponifiable fraction of a cod-liver oil.

Solutions of muscle tissue prepared in this manner were always colorless and colorless but became somewhat turbid when irradiated. Occasionally this turbidity formed when the prepared solution was stored in the refrigerator overnight, in which case filtering to the absorption cells solved the difficulty. The following procedure, however, invariably led to a clear, colorless final solution with no tendency to become turbid either on refrigeration or irradiation.

After saponification the sample was cooled, 30 cc. of water and 10 cc. of saturated sodium sulfate were added, and the flask allowed to stand 5 to 10 minutes in an ice bath. The solution was then filtered into a separatory funnel (Whatman No. 44) and the flask and filter were washed with a few cubic centimeters of a mixture of half 95% ethanol and half water, saturated with sodium chloride. The extraction was then carried out as usual.

On applications of this treatment to preparations of muscle tissue, liver tissue, and cod liver oil, it appears that such a "salting out" does not cause any appreciable loss of vitamin A.

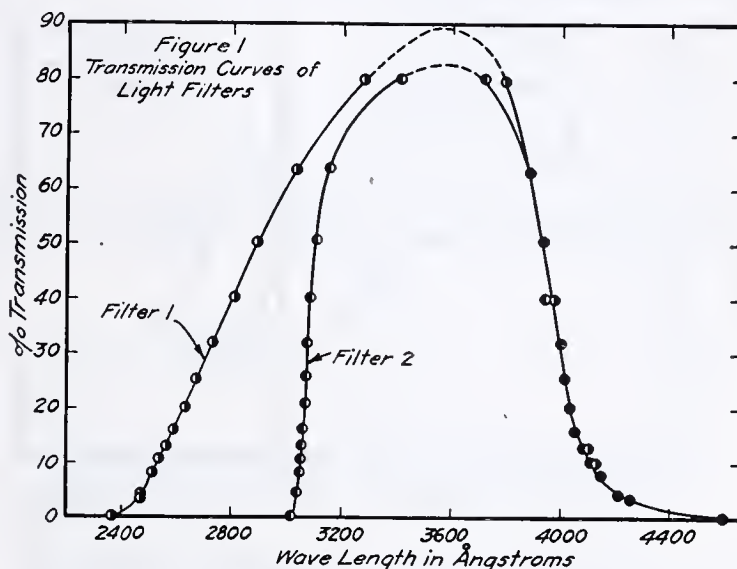
In the course of this work specific extinction coefficients were determined by three different methods—(1) direct measurement, (2) irradiated measurement, and (3) modified photometry.

The direct measurement is generally used in spectrographic work, in which case the spectrogram is taken with one absorption cell containing the solution under test and the other cell filled with pure solvent. The specific extinction coefficient is represented by the symbol  $E_{1\text{ cm.}}^{1\%}$ .

In the second case two spectrograms are taken; one a direct measurement as above, and the other a measurement of the same preparations left in the cells, the solution having been irradiated. In this case the symbol  $E_{1\text{ cm. irr.}}^{1\%}$  is used, where

$$E_{1\text{ cm. irr.}}^{1\%} = E_{1\text{ cm. (original)}}^{1\%} - E_{1\text{ cm. (residual)}}^{1\%}$$

The method of modified photometry as suggested by De (5) requires but one spectrogram, in which case both cells are filled with the solution under test, the solvent cell being irradiated before the spectrogram is taken. The symbol  $E_{1\text{ cm. mod.}}^{1\%}$  will be used in this case.



It is obvious that methods 2 and 3 should give the same specific extinction coefficient for any given solution and also a measure of those constituents of the solution which are destroyed by irradiation—i.e., vitamin A if the effect of irradiation can be made specific for the vitamin.

## EXPERIMENTAL

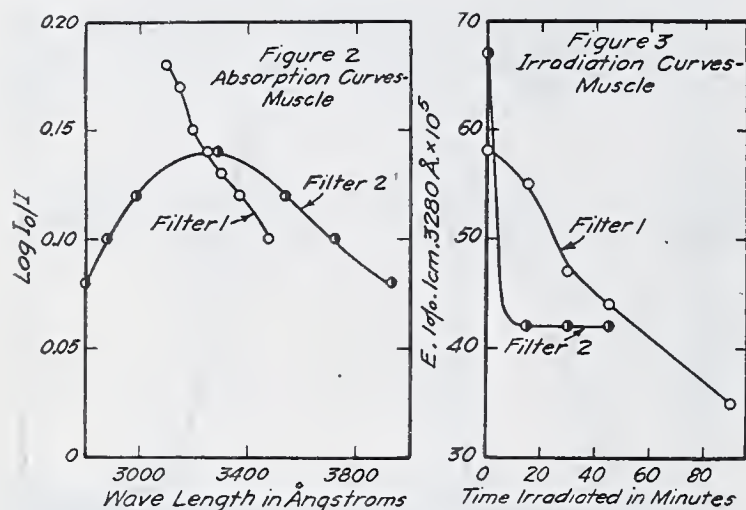
INSTRUMENT FACTOR. Specific extinction coefficients ( $E_{1\text{ cm.}}^{1\%}$ ) of the unsaponifiable fraction of U.S.P. reference oil 2, as measured ten times over a period of 9 months, ranged from 0.69 to 0.80, the mean being 0.73 with an average deviation of  $\pm 0.03$ , the instrument factor calculated therefrom being 2330.

Thus, the precision of the method when applied to oils, including changes in the oil on aging and differences introduced by the saponification and extraction processes, amounts to approximately  $\pm 4\%$ . The working factor used in potency calculations was the mean value given rounded off to 2300. Both the  $E$  value and the factor above gave satisfactory checks with other laboratories running spectrophotometric assays on the same unsaponifiable fraction and whole oil preparations.

LIGHT FILTERS AND EFFECT OF FILTERED RADIATION. In the selection of light filters to cut down the radiation of the full mercury vapor arc, two conditions should be satisfied: (1) The filter should cut out, as thoroughly as possible, the radiations in the range from 2000 to 3000 Å. (2) The remaining radiations from 3000 to 4000 Å. should be as intense as possible. It was felt that since almost all the extraneous absorption due to constituents of the fish oils other than vitamin A lies in the range from 2000 to 3000 Å., radiations of the mercury arc from 3500 Å. to longer wave lengths should have no appreciable effect on the solutions studied, and need not be considered. The two chief maxima of the oils lie generally at about 2300 and 2700 Å. These maxima completely overshadow the vitamin absorption, except



in the case of concentrates of vitamin A, and therefore are the most probable sources of change in extraneous absorption when exposed to light radiations of the proper wave length. For the irradiation studies two filters were used—filter 1: red-purple Corex A, C. G. No. 986; filter 2: C. G. No. 986 plus phthalate filter. The use of potassium acid phthalate solution as a filter for ultraviolet light was first suggested by Saunders (16). It is particularly applicable for this work since it cuts out completely all light radiations of wave lengths shorter than 3000 Å. The transmission curves of these two filters are shown in Figure 1. The transmission curves as shown do not give a true picture of the influence of each on the effective radiations of the mercury vapor arc. The effect of these curves on the absolute intensities of the ultraviolet spectrum of the low intensity quartz mercury arc, as given by McAlister (11), should be considered.



With the majority of the solutions considered throughout this work the effect of filter 1 was found to be sufficient to make insignificant those changes in extraneous absorption which occur during irradiation. With all solutions of muscle tissue and with a few low-potency preparations of liver tissue the use of filter 2 was required to obtain anything like a specific effect for vitamin A. (Filter 2 might well be used for all samples regardless of potency.) In Figure 2 are given the absorption curves for muscle preparations employing modified photometry, using filter 1 in one case and filter 2 in the other. Figure 3 shows the effects of the two filters on the rate at which the vitamin is destroyed in a preparation of muscle tissue.

From Figure 2 it will be seen that, in spite of the very low potency of this sample (less than 3 I.U. per gram), and the presence of large amounts of other absorbing constituents, the use of filter 2 produced a curve very much more similar to that of pure vitamin A than the corresponding curve obtained with filter 1. The destruction curve in Figure 3 using filter 2 shows a clean-cut decrease in absorption at 3280 Å. reaching a steady minimum value. This closely resembles the destruction curves found for other tissues, oils, and vitamin A itself. With filter 1, on the other

Table I. *E* Values and Factors for U.S.P. Oil 2

(Determined by direct and irradiation techniques)

Sample	$E_{1\%1\text{cm.}}$		Factor	
	Direct	Irradiation	Direct	Irradiation
1	0.78	0.70	2180	2430
2	0.72	0.63	2360	2700
3	0.80	0.67	2120	2540
4	0.78	0.72	2180	2360
5	0.73	0.63	2330	2700
Mean	0.76	0.67	2240	2540
Av. deviation	±0.03	±0.03	(2200)	(2500)
% av. deviation	±3.9	±4.5		

hand, the destruction curve shows no tendency to level off as it does with filter 2.

DECOMPOSITION ON IRRADIATION. The questions which first arose in the use of destructive irradiation were those which Dermer (6) expressed in his conditions 1 and 3—i.e., does the irradiation destroy vitamin A leaving no absorbing end products, and how long should the irradiation be continued to ensure quantitative destruction? The work of Neal *et al.* (14) indicates that the disappearance of absorption at 3280 Å. is accompanied by a quantitative loss of biological activity; thus the disappearance of absorption at 3280 Å. may be interpreted as indicating the destruction of the vitamin A molecule by irradiation. The practice in this investigation was to determine for each new sample which differed in nature or potency from the last considered, the time necessary to attain 100% decomposition—i.e., the irradiation time required to give an *E* value which was unchanged by further irradiation. This irradiation time was then used in assaying the remaining samples of the particular tissue or oil. For the great majority of samples the irradiation was carried out for 30 minutes, this time being used for any material which indicated complete destruction in 30 minutes or less.

In Figure 4 are given a few decomposition curves characteristic of all those obtained. A study of these curves, keeping in mind the large range of potency of the materials represented, indicates satisfaction of the two conditions stated above. The fact that the residual absorption found in samples of low potency is not due to an end product of the vitamin A decomposition, but rather to the end absorption of a constituent of the oil or tissue unaffected by the irradiation, is shown by the liver curves, which go to zero absorption even in the case of liver of lowest potency. Further proof of this fact lies with the curve of crystalline vitamin itself.

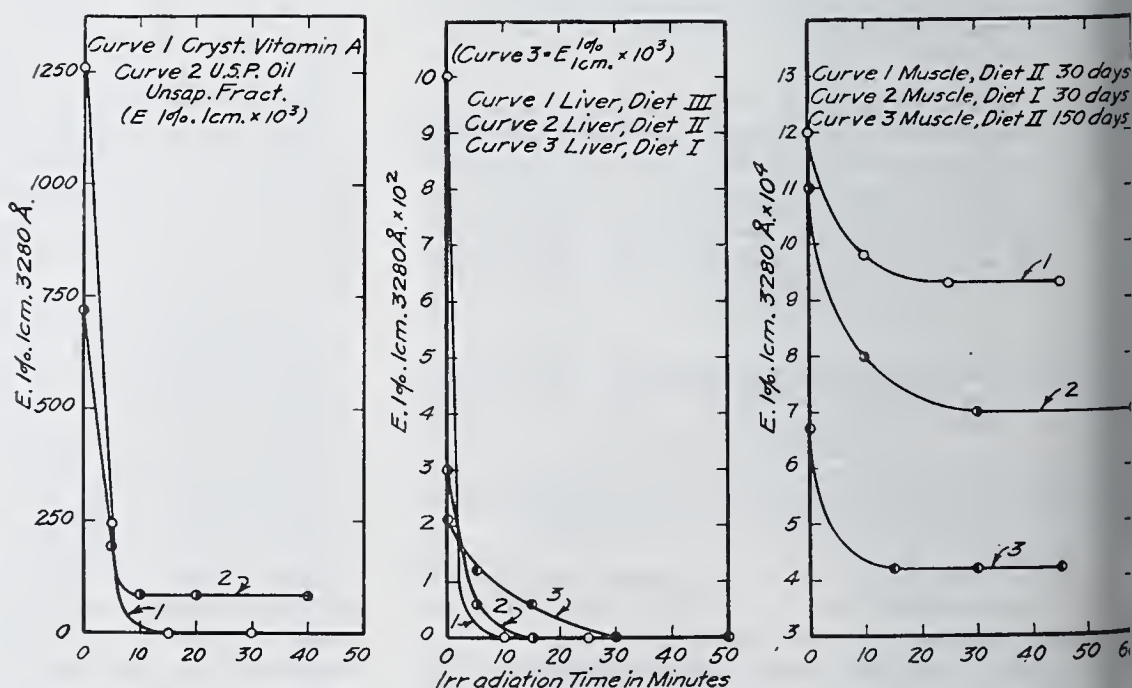


Figure 4. Effect of Irradiation on Absorption at 3280 Å.



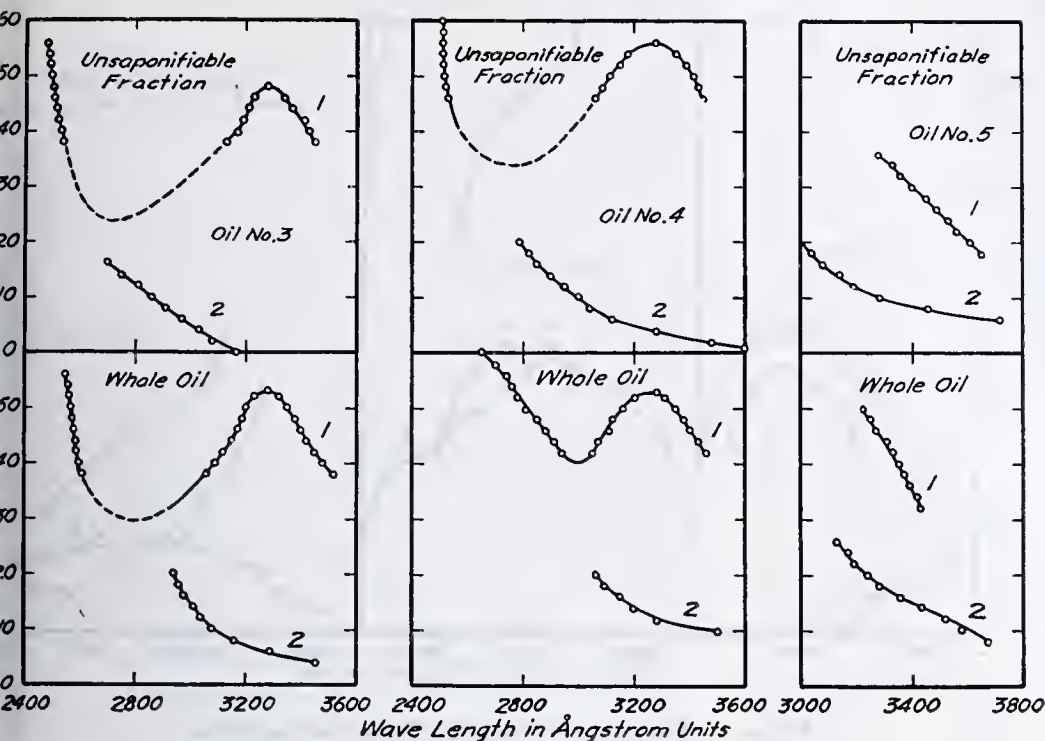


Figure 5. Absorption Curves

1. Direct 2. Irradiated

RADIATION OF FISH LIVER OILS AND CONCENTRATES. In the course of this study it was noticed that the destructive irradiation preparations of U.S.P. oil 2 never caused the  $E$  value to decrease to a value of zero. In substantiation of this, a group of U.S.P. oil preparations were measured by both the direct and irradiation methods (Table I).

The results show a distinct difference between the values of  $E_1^{1\%}$  and  $E_1^{1\%}$  cm. irradiated, which is greater than the average deviation. In view of the results of the preceding section, the determination of the instrument factor by means of  $E_1^{1\%}$  cm. would result in a value from 8 to 12% too low and would thus introduce an error of the same magnitude into all calculations of vitamin A potency, based on the factor thus obtained. In all calculations of biological potency from  $E$  values in this work, the "modified" factor of 2500 was used in place of the "direct" factor of 2300. The value of the instrument conversion factor compares very favorably with the results obtained earlier with U.S.P. oil 1. These  $E_1^{1\%}$  cm. values were  $E_1^{1\%}$  cm. = 1.21, giving a factor of 2480 (2500). In an attempt to clarify this situation, a series of determinations were made on six oils ranging in potency from 50 to 260,000 I.U. per gram, using both the direct and irradiation methods. These results are presented in Table II, and show clearly the differences in the applicability of the two methods. In so far as the relative potencies of the oils allow, the results also indicate approximate values of potency, below which the methods are in error due to extraneous absorption. As was

shown in Table I, the unsaponifiable preparation of U.S.P. oil 2 fell well below the lower limit of potency of the direct method. Further proof of this is easily obtained from the absorption curves of these oils, before and after irradiation, as plotted in Figure 5. The gradually increasing extraneous absorption of the oil with decreasing potency becomes so great in the case of U.S.P. oil 2 that it can no longer be distinguished, even in the unsaponifi-

able fraction. The destructive irradiation technique, on the other hand, can be employed with oils of potencies as low as this, even on the whole oil itself, and when saponification is carried out, can be applied to oil samples at least as low as 240 I.U. per gram.

CHANGE IN ABSORPTION CURVES PRODUCED BY IRRADIATION. The last condition, which must be satisfied in order to make the destructive irradiation technique valid as a measure of vitamin A, is that stated as condition 2 by Demarest (6)—i.e., in the course of the irradiation, substances present other than vitamin A undergo no appreciable change in their absorption at 3280 Å. That this condition is satisfied in the case of concentrates was shown by Demarest and is supported by similar work undertaken in the course of this investigation. The change in absorption curves of low-potency cod liver oils during irradiation was also found to

satisfy this condition, with the use of filter 1. In Figure 6 are given a few absorption curves for liver and muscle samples, showing the changes on irradiation and also the curves obtained by modified photometry with other samples of the same tissue. Similar curves obtained for oil samples are not given, since the validity of the above assumption in the case of these low-potency tissues is more open to question than in the case of oil preparations with potencies 300 to 1500 times as great. The potencies of the tissues in these three cases were 221, 13, and 1 I.U. per gram respectively.

In the case of Figure 6 (left) the shape of the absorption curve is very similar to that of vitamin A and falls completely to zero on irradiation. The curve obtained by modified photometry is also similar in characteristics and indicates that irradiation causes a maximum change in absorption at 3280 Å., changes at all other wave lengths being secondary. The curve obtained by calculating  $E_1^{1\%}$  cm. irradiated for curves 1 and 2 resembles 3 very closely. It seems highly probable that, in this case, changes in the absorption at 3280 Å. of constituents other than vitamin A are negligible. The potency of the liver represented by Figure 6 (center) is a great deal lower than that just considered, and the absorption of vitamin A becomes merely an inflection point on the primary curve of the tissue oil. The change of absorption is still a maximum at 3280 Å., however, as shown best by curve 3, and condition 2 is fairly well satisfied in this case also. The extraneous absorption in the case of muscle tissue is very great and the absorption of vitamin A is almost completely masked. Curve 3

Table II. Direct and Irradiated Measurements of Vitamin A Potency of Concentrates, Fish Liver Oils, and Oil Dilutions

Oil No.	Known Potency	Whole Oil		Difference, direct and irradiated %	Unsaponifiable Fraction		Difference, Whole Oil and Unsaponifiable Irradiated %
		Potency direct I.U. per gram	Potency irradiated I.U. per gram		Potency direct I.U. per gram <sup>a</sup>	Potency irradiated I.U. per gram <sup>a</sup>	
1	260,000	237,000	237,000	0	225,000	225,000	0
2	60,000	72,500	72,500	0	60,000	60,000	0
3	2,400	2,750	2,400	14	2,500	2,500	0
4 <sup>b</sup>	1,700	2,020	1,700	19	1,875	1,670	12
5 <sup>c</sup>	240	450	275	64	325	240	35
6 <sup>d</sup>	46	250	87	187	140	70	100

<sup>a</sup> I.U. per gram of original oil.<sup>b</sup> U.S.P. oil 2.<sup>c</sup> Oil 3 diluted 1-10 by weight with corn oil.<sup>d</sup> Oil 3 diluted 1-50 by weight with corn oil.



still has its maximum at 3280 Å., however, and condition 2 is again satisfied as well as might be expected for a material of such low potency. The extraneous absorption in the case of muscle preparations bears about the same relation to the vitamin absorption as it did for the low-potency oil shown in Figure 5 (right) although the potency of the oil was about 250 times that of the muscle.

#### CAROTENOID INTERFERENCE.

In determining vitamin A by a spectrophotometric measurement, the question arises as to how much of the absorption at 3280 Å. may be due to the carotenoids. In the case of butterfat the absorption at 3280 Å., which may be due to an end absorption of the carotene maxima at 4500 Å. and 4800 Å., becomes large enough to require consideration. However, in comparing the transmission curves of several prepared solutions of animal tissues with that of pure  $\beta$ -carotene, it was shown that the greatest interference by carotene at 3280 Å. amounted to less than 0.01 unit on the  $\log I_0/I$  scale.

**RECOVERY EXPERIMENTS.** In a procedure, such as is used in the preparation of the solutions of animal tissues, involving saponification, extraction, washing, and a change of solvents, two conditions must be satisfied—(1) the process must be specific in separating the constituent desired from the materials which would interfere in the quantitative measurement of that constituent, and (2) the recovery in the final solution of the substance to be determined must be as quantitative as possible.

In the case of a material like an animal tissue, condition 1 could not be completely satisfied, but, as the results show, the separation is sufficient to allow measurements to be made within a certain limit of error.

In order to test the extent to which condition 2 is satisfied, recovery experiments were carried out using supplemented and unsupplemented samples of liver and muscle tissue. The recovery in the final solutions, of the supplements added to the tissues, ranged, for the most part, from 88 to 99%. In the cases of the extremely low-potency muscle tissues, the effect of the supplement is so much closer to the precision of the measurements that calculations in terms of per cent recovery have a limited meaning. The results obtained indicated clearly, however, that the vitamin A of the sample is recovered efficiently in the final solution.

**PRECISION OF MEASUREMENTS ON ANIMAL TISSUES.** The precision of measurement, in the case of a cod liver oil, was approximately  $\pm 4\%$ . In another part of this work (10), it was found that the values obtained, with the same tissue from different but comparable test animals, varied from each other by as much as  $\pm 40\%$ . It was felt that the precision of any one measurement was much better than these results indicated, the much larger variations found being due to differences in the rates of growth and storage of the experimental animals.

In Table III are shown the results obtained when several samples of one large portion of a tissue were run on consecutive days.

It is evident that the precision of the measurements on a homogeneous sample of liver tissue is as good as that found for cod liver oils. With the muscle samples, the precision shown is also of the same order and the probable error might be expected to lie between the value given above and that caused by  $\pm 0.02$  precision

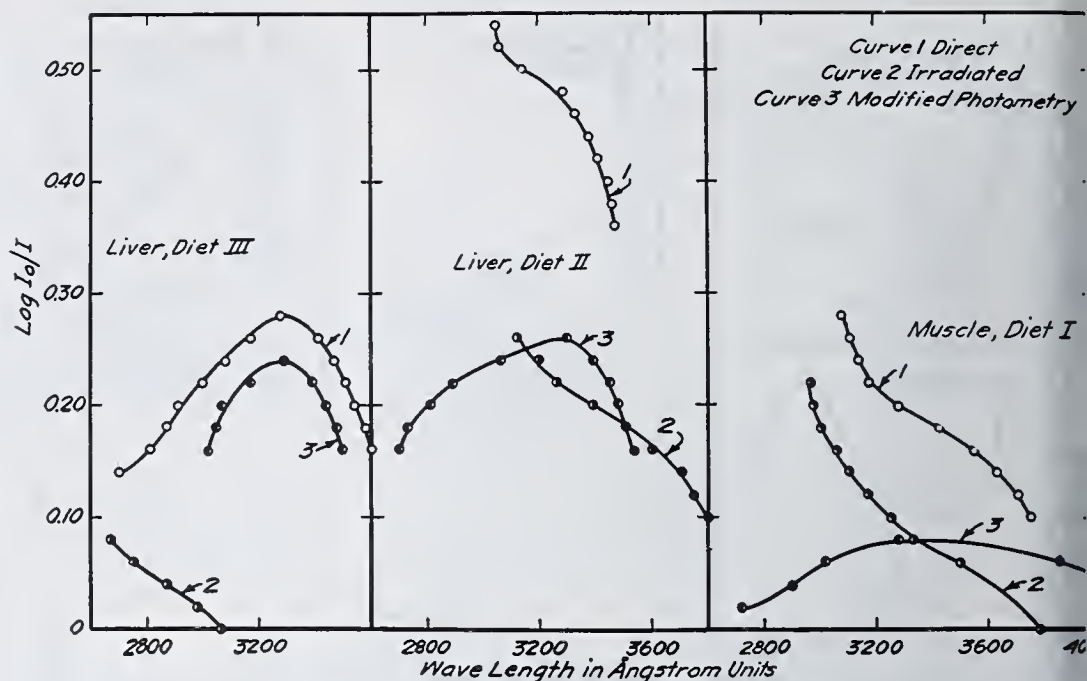


Figure 6. Changes in Absorption Curves on Irradiation

in the spectrographic measurement in terms of  $\log I_0/I$ —i.e., this case between  $\pm 5$  and  $\pm 16\%$ .

Table III. Precision of Measurement with Animal Tissues

Sample No.	Liver Potency I. U./g.	Muscle Potency I. U./g.
1	217	0.95
2	217	0.95
3	220	0.95
4	225	1.10
5	227	0.95
6	219	...
Mean	221	0.98
Av. deviation	$\pm 3.5$	$\pm 0.05$
% av. deviation	$\pm 2$	$\pm 5$

#### DISCUSSION

In order to adopt the destructive irradiation technique as a method for the quantitative estimation of vitamin A, the validity of the three assumptions previously stated must be demonstrated. On the basis of the work just described, it is considered that these three conditions have been shown to be satisfied, under conditions used, for fish liver oils and preparations of animal tissues over a very wide range of potency.

The bioassay results of Neal *et al.* (14), on unsaponifiable preparations before and after irradiation, demonstrate the complete loss of biological potency, which parallels a total loss of absorption at 3280 Å. The curves of Figure 4 show that the irradiation carried out, using the filters indicated, cause very similar decomposition curves for all types of materials studied. In the case of the liver samples the absorption at 3280 Å. is completely removed, indicating 100% decomposition of vitamin A. This is strongly supported by the destruction shown in the case of the crystalline vitamin A alcohol. On the basis of these destruction curves it may be said that condition 1 is satisfied—i.e., irradiation causes the destruction of vitamin A and the end products of the destruction have no absorption at 3280 Å. The similarity of the destruction curves and the consistent leveling out of these curves from 10 to 30 minutes, with no additional change on continued irradiation, make credible the assumption that this same quantitative destruction occurs when residual absorption is present provided irradiation is continued until the level portion of the curve is reached. A study of the absorption curves of Figures 5 and 6, observing the steady increase in extraneous absorp-



a decreasing potency, will make the validity of this assumption apparent.

The satisfaction of condition 3—i.e., that irradiation is continued long enough to ensure complete decomposition of the vitamin—is apparent from Figure 4. Pure vitamin A in cyclohexane was completely destroyed in about 15 minutes, and in 30 minutes it was eliminated in the cases of liver and of muscle.

The condition most difficult to satisfy is No. 2—i.e., the irradiation produces no appreciable change in the absorption at 3280 Å. of substances present other than vitamin A. The specificity of the irradiation for the vitamin is well demonstrated, however, for all types of materials studied, in the absorption curves of Figures 5 and 6. The primary change occurs at 3280 Å., other changes being secondary in magnitude.

That the satisfaction of these conditions is possible only by using filtered light radiations is illustrated by Figures 2 and 3. Condition 2 is definitely not satisfied when the light radiations contain wave lengths which coincide with the absorption maxima of substances present other than vitamin A. In Figure 2 the nature of the curve obtained with filter 1 could mean two things: (1) The decomposition of the vitamin is paralleled and followed by the decomposition of other constituents of the solution, or (2), as suggested by Demarest, the vitamin is screened from the effective radiations by the absorption of other constituents. The result of this second effect would be a much greater irradiation time required to obtain complete destruction of the vitamin.

The value of a method of vitamin A assay employing destructive irradiation is apparent in the application of the method to a wide range of potency, as shown in Table II. Direct measurement of the whole oil dilution has often been employed in the case of high-potency oils, common practice having been to saponify with saponification for oils with a potency of 10,000 I.U. per gram or more. Direct measurement of unsaponifiable preparations is applicable to potencies of approximately 2500 I.U. per gram or more. The applicability of the irradiation technique to whole oil preparations is even lower than this, and would probably go to values of 1000 I.U. per gram or less. Employing saponification and irradiation, the assaying of oils as low as 100 I.U. per gram or lower is possible. These limits will, of course, vary with the nature of the material assayed, and with the saponification and extraction technique used.

In the light of what has been said thus far, it would seem that  $E_{1\text{ cm.}}^{1\%}$  mod. or  $E_{1\text{ cm.}}^{1\%}$  irradiad. might be more dependable measures of vitamin A potency than  $E_{1\text{ cm.}}^{1\%}$ . The results obtained with the standard U.S.P. oil (Table I) indicate the introduction of an error of up to 12% by the assumption that  $E_{1\text{ cm.}}^{1\%}$  is a measure of vitamin A potency only. This error would carry over into calculated instrument factors and any spectrophotometric assays made on other preparations, using this factor. The similarity of the factors calculated from  $E_{1\text{ cm.}}^{1\%}$  irradiad. for U.S.P. oil 2 and  $E_{1\text{ cm.}}^{1\%}$  for U.S.P. oil 1 (0.001 in each case) suggests that the extraneous absorption in the case of the latter was small enough at 3280 Å. to give rise to no significant error in direct measurement. This is indicated, but not proved, by the results obtained with oil 3 in Table II.

It would seem possible, on the basis of the work presented, that the destructive irradiation technique may be the means of increasing the scope of the determination of vitamin A by in vitro methods. Certainly the value of  $E_{1\text{ cm.}}^{1\%}$  for preparations of materials containing vitamin A should be interpreted carefully, unless considerable research has been done on substances of comparable chemical nature, with respect to the nature of the absorption curve and the effects of irradiation.

#### ACKNOWLEDGMENT

The author wishes to take this opportunity to express his sincere gratitude to Arthur W. Thomas for his interest and helpful criticisms throughout this investigation.

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## A Funnel for Use with Standard Taper Flasks

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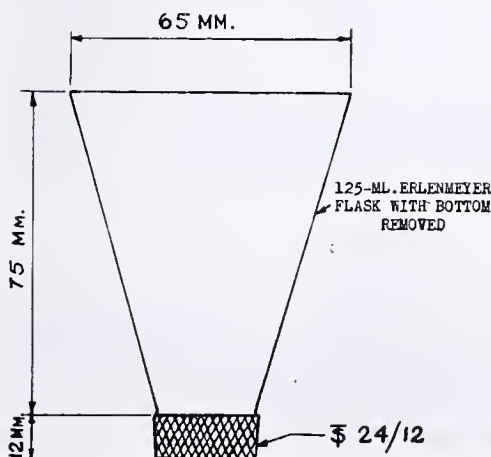
A TIME-HONORED method of adding solids to narrow-mouthed containers involves the use of a sheet of paper rolled into a funnel. Every chemist is familiar with the drawbacks of this device, and most of them have managed to spill an embarrassing amount of material in its use.

An important routine analysis in this laboratory requires the quantitative transfer of a fairly large amount of solid to a small weighing bottle having a standard taper stopper. The awkwardness of balancing a paper funnel in this operation, and the danger of loss of material in the fold of the paper, definitely suggested an improvement in this line.

The funnel shown in the illustration was accordingly constructed, and has been found highly satisfactory in practice. Because it fits firmly into the neck of the flask, the question of balancing is obviated. Since it is made of glass, the danger of particles sticking or getting lost in cracks is greatly reduced. The slope of the walls being fairly steep, solids do not tend

to pile up in the funnel.

The funnel has also been found useful in pouring large amounts of liquids and solids into other flasks, with and without ground joints, as the pouring is speeded by the steep walls and wide stem opening.





# Analytical Fractionation of Asphalts

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A procedure is described for the solvent separation of asphalts into five fractions: hexane-insolubles, hard resins, soft resins, oils, and waxes. Since all solvents employed boil above room temperatures, the method requires only ordinary laboratory glassware. All fractions are recoverable, an advantage in that they may be studied to characterize the asphalt further. The accuracy and precision of the method are discussed. Data are given to show the averages and ranges of fractions and the properties of fractions recovered from 37 oxidized and 7 straight-run asphalts, and to illustrate some applications of the method in study of asphalts.

INSIGHT into behaviors of asphalts is usually best obtainable from knowledge of the component fractions. Separation into fractions serves to characterize the asphalt. Recombinations of the fractions may be made to determine the effects of their proportions on the properties of the resulting blends. Such studies are both fundamental and practical, for they describe basic means of adjusting the fractional composition of asphalts to obtain desired action in service.

Although division into fractions with highly distinctive properties may readily be accomplished through selective solvents or adsorbents, all the laboratory methods which have been described have shown serious disadvantages.

Marcusson (14, cf. 2) in 1916 described a technique whereby the insolubles are precipitated with naphtha, and the resins adsorbed by fuller's earth. The oils are extracted from the fuller's earth with naphtha, and the resins thereafter removed by washing the earth with carbon disulfide. Various modifications of this method have been described, in all of which the resins are removed by adsorption. Important as these procedures are, it remains questionable if the resin fraction can be recovered from the clay in an unchanged condition. Uniformity of results between laboratories is difficult to achieve, even though adsorbents prepared by standard procedures are used. More recent possible methods depend upon propane as the precipitant (8, 10, 13). The primary objection to the usage of propane or of hydrocarbon gases in laboratory procedures is the notable difficulties of handling.

A method, utilizing only solvents boiling above atmospheric temperatures, which permits direct recovery and weighing of all fractions and can be practiced in ordinary laboratory equipment, was proposed by Grant and Hoiberg (6) in 1940. That procedure, while successful with asphaltic crudes of relatively low wax content, did not completely separate waxes from the resin fractions of paraffinic-base asphalts.

A revision is described here whereby this difficulty is in large part overcome. Further refinement is introduced by separating resins into two fractions, "hard resins" and "soft resins", separate knowledge of which possesses essential value in interpretation of the behavior of asphalts. Briefly, the method here presented consists in precipitation and removal of (1) the insolubles with hexane at 25° C. (77° F.), (2) the hard resins with a solution of cyclohexane-isobutyl alcohol at 37.8° C. (100° F.), (3) the waxes with an acetone-methylene chloride solution at 0° C. (32° F.), and (4) the soft resins with isobutyl alcohol at 37.8° C. (100° F.). The portion soluble in isobutyl alcohol is classed as "oils".

## PROCEDURE

Basis of analysis is a 10- to 15-gram sample, although if desired smaller equipment and more accurate weighings will permit the procedure to be followed on a 5-gram sample of asphalt. With 10 to 15 grams, weighings on the sample or the fractions derived need be carried out to only within 0.01 gram.

**VOLATILE OILS.** A loss by evaporation will occur during the analysis of road oils or asphalts which have a flash point, Cleveland

open cup, below about 232° C. (450° F.). This loss can be reported as such or the volatile oils may be removed prior to analysis by a vacuum and/or a steam distillation.

**HEXANE-INSOLUBLES.** The sample of asphalt, flux, or is weighed into a 3-liter flask, and 50 ml. of commercial m hexanes are added per gram of sample. With high-mel asphalts, and in the absence of efficient mechanical agitati heating and refluxing of the solution or addition of the asph as a powder is necessary to disperse the material in a reasona time. (The agitation provided by a Ro-Tap sieve shaker been found to disperse the asphalts rapidly.)

The solution is cooled in a water bath for 1 hour at 25° 1° C. (77° ± 2° F.) and filtered twice, with suction. The f filtration, which removes the bulk of the insolubles, is thro two sheets of filter paper, such as a Whatman No. 50 on top Sargent No. 500, in a Büchner funnel. The second filtration through a No. F sintered-glass Büchner funnel. After each filt tion, the insolubles are washed with the minimum quantity hexane which will produce clear washings. The washings added to the filtrate.

The final filtrate is distilled over a water bath until ab 150 ml. of solution remain. This is transferred to a centrif bottle (a 250-ml. Pyrex sterilizer bottle is suggested) which been previously weighed to the nearest 0.01 gram. The maining hexane is evaporated, with removal of the last traces complished under reduced pressure. The residue is weighed the insoluble content calculated.

**HARD RESINS.** To the residue in the centrifuge bottle weighed a solution of 80% isobutyl alcohol-20% cyclohex (by volume) equal to eight times the weight of hexane-solub The mixture is refluxed for 15 minutes with occasional shak to hasten solution. Periodic agitation during this time has b found necessary to obtain reliable results on resin conta especially in the analysis of asphalts of high resin conta After cooling to 65.6° ± 3° C. (150° ± 5° F.), 1.80 ml. an isobutyl alcohol solution containing 2.0% by weight sodium hydroxide are measured from a buret into the cen trifuge bottle for each 100 grams of 80% isobutyl alcohol-2 cyclohexane solution used in dissolving the petroleues. mixture is thoroughly agitated, cooled in a water bath h at 37.8° ± 0.5° C. (100° ± 1° F.), and maintained at t temperature for at least 5 minutes. The mixture is then c trifuged for 5 minutes at 37.8° ± 1.5° C. (100° ± 3° F.).

The solution of oil, wax, and soft resins is quickly decan from the centrifuge bottle through a Gooch crucible contain an asbestos mat into a tared 250-ml. Soxhlet extraction fl The bottle and filter are rinsed with 21 ml. of a solution of 8 isobutyl alcohol and 20% cyclohexane by volume which been heated to 37.8° C. (100° F.). The solvent is used three 7-ml. portions. Since the purpose is to rinse the surfa free of solution containing oil, wax, and soft resins, and further to extract the hard resins, this operation should be carried out quickly. The last portion of the wash solu which cannot be decanted is pipetted from the centrifuge bot

The resinous material collected in the Gooch crucible is was into the centrifuge bottle with hexane or light solvent naph The solvents are distilled from the bottle, with the last tra removed under reduced pressure. The residue is reported hard resins.

The oils, waxes, and soft resins are recovered under redu pressure and weighed before continuing the extraction.

**WAXES.** To the oil, wax, and soft resin residue are ad 10 parts by weight of a solvent containing 1 volume of acet to 2 volumes of methylene chloride. After as complete s tion as is possible of the residue, the mixture is cooled to 0° 0.3° C. (32° ± 0.5° F.). The precipitated wax is removed filtering under slightly reduced pressure through a Whatn No. 42 or other hard-surfaced filter paper held in a cold fun maintained at 0° ± 0.3° C. (32° ± 0.5° F.). The addit of dry ice to hexane or other light solvent provides a conveni method of maintaining the desired temperature in the c funnel. Three 7-ml. portions of the dewaxing solvent at 0° (32° F.) are used to wash the wax.

The wax is dissolved from the filter paper with hexane i the flask in which the precipitation was made and the solv distilled over a lamp bank. Final trace of the solvent is remo under reduced pressure. The weighed residue is reported wax.

The remainder of oil and soft resin solution is transferred t

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ed centrifuge bottle, recovered under reduced pressure, and ighed before continuing the extraction.

**OILS AND SOFT RESINS.** To the residue in the centrifuge tle is added isobutyl alcohol equal to six times the weight the oils and soft resins. The mixture is refluxed for 15 min- es. After cooling to  $65.6^{\circ} \pm 3^{\circ} \text{C}$ . ( $150^{\circ} \pm 5^{\circ} \text{F}$ .), 0.50 ml. of isobutyl alcohol solution containing 2.0% by weight sodium droxide is measured from a buret into the centrifuge bottle each 100 grams of isobutyl alcohol used. The cooling and atrifuging procedure at  $37.8^{\circ} \text{C}$ . ( $100^{\circ} \text{F}$ .) is then followed as the separation of the hard resins.

The oil solution is decanted from the centrifuge bottle through asbestos mat held in a Gooch crucible into a weighed extrac- n flask. The centrifuge bottle and filter are washed with ce 7-ml. portions of isobutyl alcohol heated to  $37.8^{\circ} \text{C}$ . ( $100^{\circ} \text{F}$ .).

The residue is washed from the crucible with hexane into centrifuge bottle. The solvent is distilled from the centrifuge tle under reduced pressure and the residue reported as the percentage of soft resins.

The oils are separated from the solvent under reduced pressure d weighed, or if their recovery is not desired they may be re- rted by difference.

**SOLVENTS.** Mixed hexanes (from Phillips Petroleum Com- ny, Special Products Division, Bartlesville, Okla.), of nil saturate content, and with an initial boiling point of not less an  $54^{\circ} \text{C}$ . ( $130^{\circ} \text{F}$ .) and a dry point not greater than  $71^{\circ} \text{C}$ . ( $160^{\circ} \text{F}$ .) were employed to precipitate the insolubles.

The isobutyl alcohol (from Eastman Kodak Company, emical Sales Division, Rochester, N. Y.), Eastman grade, emed in the range of  $105\text{--}108^{\circ} \text{C}$ . ( $221\text{--}226^{\circ} \text{F}$ .).

The cyclohexane (Eastman), with a density at  $25^{\circ}$  ( $77^{\circ} \text{F}$ .)  $0.77 \pm 0.01$  gram per ml., boiled in the range of  $78\text{--}81^{\circ} \text{C}$ . ( $172\text{--}176^{\circ} \text{F}$ .). While the Eastman grade was preferred, selected tches of the practical grade were found to give essentially e same yields of hard resins. Use of other batches of the actical grade of higher density lowered the yield of resins. The methylene chloride (Eastman), practical grade, boiled the range of  $39\text{--}43^{\circ} \text{C}$ . ( $102\text{--}109^{\circ} \text{F}$ .).

The acetone (Eastman), practical grade, boiled in the range  $55\text{--}57^{\circ} \text{C}$ . ( $131\text{--}135^{\circ} \text{F}$ .).

**CHARACTERIZATION OF RECOVERED FRACTIONS.** Knowledge the character of the precipitated fractions is of importance relating the composition to properties and behavior of asphalts. ethods which may be followed to determine the refractive dex and specific gravity, and to calculate the specific dispersion d refractive intercept have been described (6, 7).

With the high-melting hard resin fraction difficulty was had reading an Abbe refractometer. An approximate procedure lowed was to estimate the refractive index and drum reading r blends of low-viscosity lubricating oils and the resins, and en to extrapolate the data to zero oil content.

The melting point of the wax fractions was determined by e procedure recorded by Knowles and Levin (13).

The viscosity of the oil fractions was determined with micro- scometers of the type described by Cannon and Fenske (5). Pour points of the oils were determined in an  $8 \times 50$  mm. test be one-third full of the oil fraction, since an insufficient ount of this fraction was available for filling the standard S.T.M. tube. The test tube was closed by a cork carrying an S.T.M. cloud and pour test thermometer. The test tube was ted through a stopper into a standard cloud and pour point st jar and the annular space filled with a light lubricating oil. he assembly was heated in a water bath to about  $49^{\circ} \text{C}$ . ( $120^{\circ} \text{F}$ .), moved to a standard cooling bath, and observation made as quired by the procedure described under A.S.T.M. Designation 97-39.

**EQUIPMENT.** The arrangements used in the centrifuging and tering of wax, and in the evaporation of small volumes of olvents, are discussed only for the sake of illustration. Many odifications are possible.

A G.E. centrifuge, Size 1, Type C, was heated by blowing air through an opening in the cover. The air was heated by passage ver a 500-watt electric element held in an insulated brass yinder 9 cm. in inside diameter  $\times$  38 cm. Cold air was by- assed into the heated stream to maintain the temperature of he air leaving the centrifuge through the bottom drain opening t  $38.3^{\circ} \text{C}$ . ( $101^{\circ} \text{F}$ .), at which temperature the whirling bottles ere found to remain at  $37.8^{\circ} \pm 0.5^{\circ} \text{C}$ . ( $100^{\circ} \pm 0.5^{\circ} \text{F}$ .). When temperature of the outlet air rose above  $38.3^{\circ} \text{C}$ . ( $101^{\circ} \text{F}$ .),

slight lifting of the cover of the centrifuge was found to admit sufficient air to reduce the temperature the desired extent. The centrifuge was operated at about 1800 r.p.m.

The cold funnel was constructed from the upper half of a carbon disulfide can, 12 cm. in inside diameter  $\times$  8.5 cm. A lip was soldered on to reduce the opening of the can to a diameter of 8 cm., leaving, however, a notched-out portion for the introduction of dry ice and a thermometer. A 9-cm. outside diameter glass funnel, held in place by a rubber stopper set in the outlet for the can, could then fit tightly to the lip to prevent the cooling liquid from splashing into the funnel. The outside of the can was insulated.

A lamp bank was constructed as a convenience in evaporat- ing small amounts of light solvents, as hexane and the wax precipitating solvents. Seventy-five-watt light bulbs were enclosed in a 9.5-cm. diameter  $\times$  21-cm. metal cylinder, pro- vided with a screened top and arranged in banks of 4.

#### DEVELOPMENT OF REVISED PROCEDURE

The original procedure (6) was based on the removal of the insolubles with pentane, precipitation of the total resin fraction at  $54.4^{\circ} \text{C}$ . ( $130^{\circ} \text{F}$ .) with isobutyl alcohol, and separation of the waxes from the oils at  $0^{\circ} \text{C}$ . ( $32^{\circ} \text{F}$ .) with a solvent consisting of 60% acetone and 40% methylene chloride by volume. These conditions were suitable in selectively precipitating fractions from naphthenic-base asphalts. Trials with paraffin-base asphalts of high wax content showed, however, that an appreciable por- tion of the wax was precipitated along with the resin fraction. Two obvious changes were possible to improve the separation of the resin and wax fractions. Actually, a combination of these modifications was devised.

The solvent used to precipitate the resin fraction could be modified to become a better solvent for wax, providing that the resin fraction would still be precipitated. As a less practical equivalent, the temperature at which the resins were precipitated  $54.4^{\circ} \text{C}$ . ( $130^{\circ} \text{F}$ .) could be raised.

The wax could be precipitated from the resin-wax-oil mix- ture if a dewaxing solvent with a high solubility for resins could be found.

**REVISION OF DEWAXING PROCEDURE.** Many of the un- settled discussions of the influence of wax on service behavior of asphalts are the result of the wide range of properties exhibited by hydrocarbons which can properly be included under the descriptive term "wax". The determined wax content of a given asphalt can vary greatly, dependent on the method em- ployed in the determination. Both types of paraffins, those which are solid or liquid at ordinary temperatures, or only the higher melting compounds may be recovered. A selection of conditions which will precipitate the fraction most informative of the composition and behavior of asphalts is desirable. Some of the phenomena are listed below which led to the belief that knowledge of only the higher melting waxes would usually be of greater importance.

Blown asphalts from a light Arkansas asphalt, flux A, were about 30 points higher in penetration at  $25^{\circ} \text{C}$ . ( $77^{\circ} \text{F}$ .) than an asphalt of the same melting point blown from another light Arkansas asphalt, flux B. Determinations on both fluxes at  $-17.8^{\circ} \text{C}$ . ( $0^{\circ} \text{F}$ .) yielded 16% of a wax liquid at room temperature. On the other hand, determinations at  $0^{\circ} \text{C}$ . ( $32^{\circ} \text{F}$ .) by the method described herein showed flux B to contain 3.0% and flux A, 10.5% of a wax solid at room temperature. The con- tents of the hexane-insolubles and the resins from the blown as- phalts of both fluxes were very nearly identical. The higher content of high melting wax of flux A was apparently effective in decreasing the homogeneity of the asphalt, thereby raising the penetration.

As another effect, the blown asphalt from flux A, which has a distinctly "waxy" appearance, stained paper to a dark olive color, whereas the blown asphalt from B was normal in ap- pearance and did not stain paper.

Experiences with poor adhesion and the noncuring of road oils have also pointed to the content of high-melting waxes as of greater importance than the total paraffin content. It is thought that as the oil fraction becomes heavier during service the higher melting waxes are precipitated to form a nonhomo- geneous and noncohesive binder. Road oils of this type that



Table I. Dewaxing Trials with Various Solvents

Dewaxing Solvent <sup>b</sup>	Blank on Cylinder Stock <sup>a</sup>				Determination on 40% Wax-60% Cylinder Stock Blends						
	Solvent ratio <i>G./g. cylinder stock</i>	Temp. of ppt.		Cylinder stock insoluble  %	Solvent ratio  <i>G./g. blend</i>	Temp. of ppt.		Wax found  %	Wax found less blank  %	Error  %	Refrac- tivity of wax <i>n<sub>D</sub><sup>25</sup></i>
		° C.	° F.			° C.	° F.				
Methyl isobutyl ketone	22.8	-17.8	0	4.3	23.5	-17.8	0	39.1	34.8	5.2	1.48
Methyl isobutyl ketone	23.0	0.0	32	1.2	23.4	0.0	32	26.9	25.7	14.3	1.48
35% acetone, 65% benzene	10.1	0.0	32	2.5	10.0	0.0	32	25.9	23.4	27.0	1.49
60% acetone, 40% methylene chloride	9.9	0.0	32	64.5	10.0	10.0	50	76.0	..	..	1.48
50% acetone, 50% methylene chloride	9.9	0.0	32	47.5	9.6	10.0	50	68.2	..	..	1.48
33.3% acetone, 66.7% methylene chloride	10.0	0.0	32	1.0	10.0	0.0	32	39.1	38.1	1.9	1.47

<sup>a</sup> Refractive index at 50° C. ( $n_D^{50}$ ) = 1.512. <sup>b</sup> Proportions given by volume.

fail to set often reduce by the A.S.T.M. method to a 100 penetration residue that is grainy, noncohesive, and waxy in appearance.

With these effects of waxes in mind it was decided to include with the oil fraction that portion of the paraffin fraction which is liquid at ordinary temperatures. The properties of this fraction would probably be sufficiently unique to be reported separately. However, this would introduce an additional complexity in the method which should not generally be necessary, since the average properties of the oils can be indicated by physical measurements, such as specific dispersion. However, if the composition is to be determined in connection with a study of the properties of the asphalt at low temperatures, it seems likely that more exact knowledge of the content of the lower melting paraffins would be important, since at low temperatures these would presumably also be solid in the asphalt. In such a case, separation could be accomplished by further dewaxing of the oils, or the wax could be precipitated by the procedure recommended herein but at a temperature lower than 0° C. (32° F.)

**Selection of Dewaxing Solvent.** Relatively few solvents have been discussed in the literature with regard to their selective solubility for highly resinous oils and for high-melting petrolatum waxes. Usually the solvents have been investigated and classified according to their solubility for a dewaxed refined oil and refined paraffin. An exception is the acetone-methylene chloride solutions used in separating waxes from petroleum and its lubricating fractions (11). Acetone-benzene solutions are also used commercially to dewax untreated lube oil stocks (12). Hexone (methyl isobutyl ketone) has been reported to be satisfactory for the quantitative dewaxing of propane-soluble heavy lube oils (13).

The results of dewaxing trials with these three solvents, all of which have properties suitable for laboratory dewaxing procedures, are shown in Table I. The heavy cylinder stock of 245 seconds Saybolt Universal viscosity at 98.9° C. (210° F.) used in the trials was obtained by propane-deasphaltizing an asphaltic flux. Wax was recovered from a portion of the stock by dewaxing at -17.8° C. (0° F.) with 4 volumes of a solvent containing 35% acetone and 65% benzene by volume. The recovered wax was reprecipitated at -17.8° C. (0° F.), using 10 volumes of solvent to 1 volume of wax, and then cleaned by mild acid and clay treatment. The final wax with a refractive index at 50° C. (122° F.) of 1.478 melted at 63.3° C. (146° F.).

Results indicate that of the three best solvents for the cylinder stocks, the 33.3% acetone-66.7% methylene chloride solution recovered the wax at 0° C. (32° F.) with the smallest error. The refractive index of the wax precipitated by this solvent was the same as that of the wax added to the cylinder stock, showing that no resinous material was precipitated from the heavy cylinder stock.

**Content of High Melting Waxes in Petrolatum.** Two petrolatums were dewaxed at 0° C. (32° F.) with various ratios of the 33.3/66.7% acetone-methylene chloride solvent. The properties of the petrolatums, the variation in wax content with solvent ratio, and the properties of the separated wax and oils are given in Table II. The results are believed to be indicative of the variation which would be expected in wax recovery from an asphalt with a change in solvent ratio. This is especially so

since petrolatums I and II were derived from residual oils and therefore, contained the high-melting waxes normally present in asphalts.

The variation in wax content with the solvent ratio, calculated from the weights of solvents used and wax found, is shown graphically by Figure 1. The content of wax precipitated ratios above 80 decreases only slightly with increase in ratio. Since in the usual asphalt analysis the ratio of the acetone-methylene chloride solvent to the determined wax content is near or above 100, the type and amount of wax recovered should be essentially independent of the exact ratio established by the procedure.

The oils recovered, which at the higher ratios represented a substantial portion of the petrolatum, were highly paraffinic, shown by refractive indices. However, for reasons previously discussed, it is believed that these oils properly belong in the fraction because of their liquid character at ordinary temperatures. Dewaxing at 0° C. (32° F.) with the acetone-methylene chloride solvent thus appeared to be satisfactory for the purpose in mind.

**MODIFICATION OF SOLVENT TO PRECIPITATE RESIN FRACTION.** Since the results with the acetone-methylene chloride solvent (Table I) had indicated that the wax determination could be run on a highly resinous stock without coprecipitation of the resins, a solvent to increase the solubility of wax could be added to the precipitant for resins. Two solvents, toluene and cyclohexane, which boil in the desired range and are fairly good solvents for wax, were blended with the isobutyl

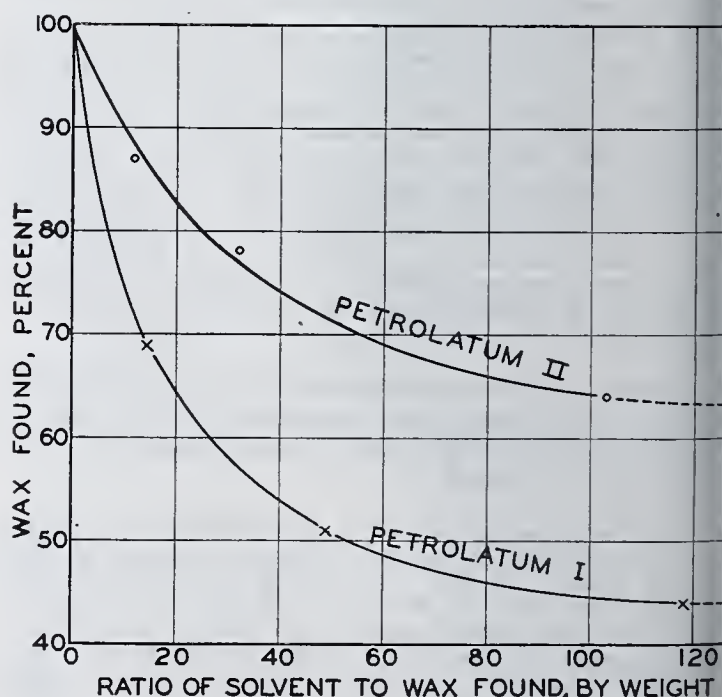


Figure 1. Dewaxing of Petrolatums at 0° C. (32° F.)



hol. Precipitations were completed with the alcohol vents on asphalts from which the pentane-insolubles had viously been precipitated.

The contents of resins obtained are shown in Table III. Since resin content determined according to the original procedure been 51 and 78% on the blended asphalt and flux, respectively, reduction in yield upon adding the toluene or the cyclohexane very marked. Nevertheless, the wax precipitated at 0° C. ° F.) with the acetone-methylene chloride dewaxing-solvent a refractive index low enough to indicate but little resin tamination. The wax recovery was slightly higher from the resin-oil-wax mixtures soluble in the toluene-isobutyl hol solvents, but the wax was lower in melting point.

with a probable increase in solubility for wax in the isobutyl alcohol solutions.

**PRECIPITATION OF RESINS.** *Addition of Cyclohexane to Isobutyl Alcohol.* Cyclohexane was selected in preference to toluene for addition to the isobutyl alcohol. The addition of 20% by volume improved the solvency of the alcohol for wax to about the same extent as did toluene. A definite advantage was the precipitation of a larger fraction of resins by the cyclohexane solution, as shown by data of Table II. Unless the resin fraction represents about 10% or more of the asphalt, characterization tests are difficult and changes in the fraction which occur in service or processing cannot readily be followed. The lower boiling point of cyclohexane would also make easier the removal of the last traces of solvent.

*Separation of Soft Resins.* Since addition of cyclohexane to the isobutyl alcohol markedly increased the solvency of the alcohol for resins, the fraction of the asphalt remaining after dewaxing would be highly resinous. A further separation of resins from the oils was indicated. Preliminary trials showed that precipitation at 37.8° C. (100° F.) with isobutyl alcohol removed a large part of the remaining resin fractions. To distinguish between the two resin fractions, the precipitate formed with the 20% cyclohexane-80% isobutyl alcohol solution was designated as hard resins, and the precipitate with the isobutyl alcohol, soft resins.

*Precipitating Agents for Resin Fractions.* With the change from pentane to hexane to remove the insolubles, difficulty was found in precipitating the hard resins when ammonium hydroxide was used as the flocculent. Incomplete settling of the resins was obtained even after prolonged centrifuging. With the use of hexane a fraction was apparently shifted from the insolubles to the hard resins which

was very difficult to flocculate. An increase in the concentration of ammonium hydroxide was not desirable, since additional water to lower the solubility of wax would then be present. Consequently, other agents were tried. A chemical used to break crude oil-water emulsions, Visco 242A, and a wetting agent, Aerosol OT 100%, were unsuccessful. Sodium hydroxide in a concentration of 0.03% by weight in the cyclohexane-isobutyl alcohol solution was found to settle the hard resin fraction rapidly. The addition of 0.01% of sodium hydroxide to the isobutyl alcohol solution was sufficient to flocculate the soft resin fraction. The added caustic is probably precipitated with the wax fraction and in the average analysis would increase the percentage of wax by about 0.2%.

#### ANALYSIS OF ASPHALTS

**ASPHALTS OF HIGH PETROLATUM CONTENT.** The recovery obtained of fractions from asphalts to which known amounts of high-melting petrolatum had been added is shown by Table V. The calculated compositions of the asphalts as given are based upon determined compositions of the asphalt blanks and of the oil and wax contents of the added petrolatum. The wax contents of the petrolatums were selected from the graph of Figure 1 at the actual dewaxing ratio employed in the analysis, rather than by assigning an average wax content to the petrolatum at an average solvent ratio. The difference between the calculated and experimental contents of wax in the asphalts then is a direct measure of the recovery of wax from the asphalt and does not include the effect of change in dewaxing solvent ratio. Actually, unless the wax content is high, above about 8%, the ratio is in the range wherein a change will have only a slight effect on wax recovery. Four asphalts listed in Table V, which contained

**Table II. Wax Content of Petrolatum at 0° C. (32° F.)**

(At various ratios of 33.3% acetone-66.7% methylene chloride solvent)						
Properties of petrolatum						
melting point, ° C.	I			II		
melting point, ° F.	62.0			72.5		
density at 65° C., g./ml.	143.5			162.5		
refractive index at 65° C. ( $n_D^{65}$ )	0.842			0.834		
specific dispersion	1.466			1.464		
refractive intercept	99			99		
and wax content of petrolatum	1.044			1.047		
ratio of solvent to petrolatum, by wt.	10	25	52	10	25	66
ratio of solvent to wax found, by wt.	14.5	49	118	11.5	32	103
Wax found, %	69	51	44	87	78	64
Oil found, %	31	49	56	13	22	36
Total	100	100	100	100	100	100
Properties of wax						
refractive index at 65° C. ( $n_D^{65}$ )	1.470	1.462	1.461	1.468	1.464	1.462
melting point, ° C.	...	67.2	68.9	...	75.0	75.0
melting point, ° F.	...	153	156	...	167	167
Properties of oil						
refractive index at 25° C. ( $n_D^{25}$ )	1.487	1.485	1.483	1.494	1.491	1.486
ur point, ° C.	...	12.8	15.7	...	21.1	26.7
ur point, ° F.	...	55	60	...	70	80
appearance of oil	...	Reddish brown	Reddish brown	...	Very dark brown	Very dark brown

**SOLUBILITIES OF PETROLATUM.** The solubilities of petrolatum the cyclohexane- and toluene-isobutyl alcohol solvents and pentane and hexane are shown by Table IV. The two petrolatums used in the trials were the same as previously de- ped, and their properties are given in Table II. The ratios olvents to waxes were selected to correspond to ratios which nally occur during separation of the fractions from asphalts rding to the procedure developed herein. Column 5 lists ulated concentrations of wax in an asphalt which would espond to these ratios. For the trials with the isobutyl hol solutions the calculations were based upon an asphalt aining 15% of insolubles and the use of 8 parts by weight olvent per part of insoluble free asphalt.

he solubility of the petrolatums was found to be appreciably r in pentane than in hexane. Consequently, hexane ind of pentane was adopted to determine the insoluble content. he solubility of petrolatum I in the isobutyl alcohol solu- s was high, above 98%. Petrolatum II, of higher melting t, was less soluble, with 92% dissolved at a solvent ratio valent to that in an asphalt containing 7.3% of wax. No eciable increase in solubility of II was found upon increasing cyclohexane content of the isobutyl alcohol from 20 to 22.5% substituting 20% of toluene for the cyclohexane.

With 92% solution of petrolatum II the wax content found ld be 6.4% instead of the correct value of 7.3%. The other ion, 0.9%, of wax would be partially precipitated by the ne, the remainder with the hard resins. Since the solu- y of wax would be increased during actual analyses by the ence of resins and oils in the solvent, it was decided that fur- studies could best be made on asphalts containing known ents of wax. Also, as described below, the use of an aqueous onia solution as a flocculent for the resins was discontinued



Table III. Resin Precipitating Trials with Various Solvents

Asphalt	Resin Precipitating <sup>a</sup> Solvent	Ratio of Solvent Added <sup>b</sup>	Temp. of Ppt.		Hard Resins Found %	Wax Added Plus Blank %	Wax Found %	Melting Point		Refractive Index of Wax, $n_D^{25}$
			° C.	° F.				° C.	° F.	
Blended	15% toluene	8	37.8	100	12.1	7.8	7.6	54.4	130	1.482
Blended	85% isobutyl alcohol									
Blended	20% toluene	8	37.8	100	6.0	7.8	8.4	55.0	131	1.482
Blended	80% isobutyl alcohol									
Blended	20% cyclohexane	8	37.8	100	15.8	7.8	7.4	62.2	144	1.489
Blended	80% isobutyl alcohol									
Flux	20% cyclohexane	6.8	37.8	100	11.4	...	8.0	65.6	150	1.487
Flux	80% isobutyl alcohol									
Flux	20% cyclohexane	6.8	40.6	105	8.3	...	7.6	66.1	151	1.484
Flux	80% isobutyl alcohol									

<sup>a</sup> Proportions given by volume.<sup>b</sup> Wt. of solvent divided by wt. of pentane-insoluble free asphalt.

from 10.9 to 14.3% of wax, were dewaxed at solvent ratios of from about 70 to 40, respectively. If calculated values for wax had been based on an assumed ratio of 100, the values would have been lower by 0.7 to 1.5%, respectively. Then comparisons with the experimental values would have shown the recovery of wax higher than was actually the case.

An average difference of +0.4% was found between the calculated and experimental values for hexane-insolubles, with a maximum difference of 1.8%. The larger differences were shown in analysis of asphalts to which petrolatum II had been added. Precipitation of waxes probably occurred. The average wax content of the asphalts was high, 9.7%, and solubility trials (Table IV) had shown that petrolatum I or II would not be completely soluble in hexane at such concentrations. Also, possibly, the weight of insolubles precipitated was increased because of the increased paraffinicity of the hexane-asphalt solution, owing to the large weight of petrolatum contained in the asphalt. This effect should be small, however, because of the large volumes of hexane used.

The recovery of resins from the petrolatum-asphalt blends was higher by an average of 1.8 and 2.4% than that calculated for the hard and soft resins, respectively. The increase in these fractions could be due to coprecipitation of waxes from the petrolatums and/or to precipitation of fractions wider in molecular weight range because of the greater paraffinicity of the precipitating mixture caused by the high petrolatum content. Thus, a portion of the soft resins would be precipitated with the hard resins and a portion of the higher molecular weight oils with the soft resins.

Since the recovered wax accounted for about 93% of the calculated wax values, and an additional 4% was presumably precipitated with the hexane-insolubles, it was evident that the paraffinicity of the decresinating solvents was largely responsible for the increased yield of hard resins. With the soft resins there was a possibility that some of the large content of paraffinic oils contained in the added petrolatum were precipitated by the isobutyl alcohol at 37.8° C. (100° F.). As an indirect check, recovery of the soft resins was made with solvents having

greater solubility for paraffins using isobutyl alcohol containing 5 and 10% of cyclohexane in analyses 8 and 9 of Table V, respectively. The yield of resin was again greater than calculated by about the same amount, which should not have been the case if paraffins were precipitated.

Some resin contamination of the wax was indicated by the values for specific dispersion which were greater than 1. The melting points of the recovered wax, with one exception, did not agree with those of

added wax. Melting points on five of the recovered waxes were lower by 3° to 12° C. (5° to 22° F.), while on three the values were greater by more than 13° C. (24° F.). The lowering of melting points of the waxes is probably caused by the previous precipitation of a small portion of higher melting waxes with the insoluble hard resins and by the presence of minor amounts of resins and oils in the waxes. The increase in melting point of three of the waxes could possibly be attributed to coprecipitation of very high melting resins. However, an analysis of blended asphalt A containing 16.2 grams of petrolatum I per 100 grams of asphalt showed no precipitation of the wax at -6.7° C. (20° F.) did not show an increased precipitation of resins: A yield of 9.0% of a wax with specific dispersion value of 111 and melting at 64° C. (147° F.) was obtained. Maintaining of a dewaxing temperature of 0° (32° F.) or above is thus apparently not critical in so far as precipitation of resins with wax is concerned.

ACCURACY. The differences between the calculated and experimental values, shown in Table V, are low for a method dependent upon batch solvent separations. Since there is no definite dividing line between the resins and oils, the differences shown are not of great significance except to indicate that with asphalts of high wax content there is a shift in the line of separation. The accuracy of recovery of the insolubles and wax is sufficiently high for most studies which might be made.

Table IV. Solubility of Petrolatum in Various Solvents

Solvent <sup>a</sup>	Petrolatum	Ratio Solvent to Petrolatum Ml./g.	Ratio Solvent to Wax Content of Petrolatum Ml./g.	Calculated Wax Content of Asphalt Based on Solvent-Wax Ratios %	Temp. of Test		Petrolatum Found Soluble %
					° C.	° F.	
Pentane	I	686	1560	3.2	25	77	100.0
Pentane	I	344	784	6.4	25	77	98.0
Pentane	II	1280	2000	2.5	25	77	95.0
Pentane	II	439	685	7.3	25	77	93.0
Hexane	I	686	1560	3.2	25	77	100.0
Hexane	I	344	784	6.4	25	77	99.0
Hexane	II	1280	2000	2.5	25	77	99.0
Hexane	II	439	685	7.3	25	77	94.0
20% cyclohexane		G./g.	G./g.				
80% isobutyl alcohol	I	93.4	212	3.2	37.8	100	99.2
20% cyclohexane							
80% isobutyl alcohol	I	46.7	106	6.4	37.8	100	98.2
20% cyclohexane							
80% isobutyl alcohol	II	174	272	2.5	37.8	100	96.2
20% cyclohexane							
80% isobutyl alcohol	II	59.6	93.1	7.3	37.8	100	92.2
22.5% cyclohexane							
77.5% isobutyl alcohol	I	93.4	212	3.2	37.8	100	99.0
22.5% cyclohexane							
77.5% isobutyl alcohol	I	46.7	106	6.4	37.8	100	99.0
22.5% cyclohexane							
77.5% isobutyl alcohol	II	174	272	2.5	37.8	100	96.0
22.5% cyclohexane							
77.5% isobutyl alcohol	II	59.6	93.1	7.3	37.8	100	92.1
20% toluene							
80% isobutyl alcohol	I	80.8	184	3.7	37.8	100	99.6
20% toluene							
80% isobutyl alcohol	II	114	179	3.8	37.8	100	94.7

<sup>a</sup> To all isobutyl alcohol-solvent blends 0.25% by volume of 27% NH<sub>4</sub>OH solution was added. This solution was used as flocculator for resins in original procedure.



Table V. Recovery of Fractions from Asphalts Containing Added Petrolatum

(Results based on average values from two duplicate determinations)

Sample No.	Asphalt	Petrolatum Type	Added G./100 asphalt	Calculated Values					Difference between Calculated and Determined Values					M. P. of Added Wax		M. P. of Wax Found		Specific Dispersion of Wax Found × 10 <sup>4</sup>
				H.I.	H.R.	S.R.	O.	W.	H.I.	H.R.	S.R.	O.	W.	° C.	° F.	° C.	° F.	
G./100 g.					G./100 g.													
Blended A	I	16.2	9.5	16.6	33.2	33.5	7.2	+0.1	+3.0	+2.9	-5.4	-0.3	69	156	82+	180+	120	
Blended B	II	12.9	9.3	13.1	35.9	33.7	8.0	+1.1	-0.1	+1.7	-2.3	-0.4	75	167	75	167	109	
Paving, 98 penetration	I	29.1	11.3	30.0	22.2	22.1	14.3	+0.9	+3.5	+4.1	-6.6	-2.1	67	152	60	141	130	
Paving, 98 penetration	II	17.7	12.4	33.0	24.4	16.0	14.3	+1.8	+3.0	-0.1	-3.2	-1.9	75	167	64	148	128	
Oxidized light <sup>a</sup> flux	I	12.8	30.6	8.1	16.0	37.6	7.7	-0.3	+1.8	+3.1	-4.0	-0.7	68	155	65	149	105	
Gulf Coast SC- 6 road oil	I	29.1	11.8	7.1	10.4	57.3 <sup>b</sup>	10.9	-0.2	-0.7	+4.3	-4.0	-0.2	68	155	61	141	117	
Mexican SC-6 road oil	II	17.7	16.0	10.3	9.9	50.3 <sup>c</sup>	11.8	+0.6	+1.4	-0.2	-2.7	+0.5	75	167	63	145	128	
Blended A	I	16.2	9.9	16.2	27.8	39.7	6.4	-0.5	+2.1	+2.1	-3.4	-0.4	69	156	82+	180+	118	
Blended A	I	16.2	9.5	16.5	20.8	46.3	6.8	0.0	+2.0	+3.6	-4.1	-0.5	69	156	82+	180+	119	
Av. difference								+0.4	+1.8	+2.4	-4.0	-0.7						

S.P. (R. and B.) 95° C. (203° F.), penetration at 25° C. (77° F.) 23.

Loss of volatile oils by evaporation during analysis, 2.5%.

Loss of volatile oils by evaporation during analysis, 1.7%.

Blend of 5% cyclohexane, 95% isobutyl alcohol by vol. used to ppt. soft resins.

Blend of 10% cyclohexane, 90% isobutyl alcohol by vol. used to ppt. soft resins.

Table VI. Precision of Method

Deviation from average of two determinations run on 60 different samples of asphalts, grams per 100 grams of original sample)

	Average Deviation	Maximum Deviation	Percentage of Samples on Which Deviation Was Greater than ±0.50
Exane-insolubles	±0.28	±0.9	20
Hard resins	±0.31	±1.1	15
Soft resins	±0.32	±1.2	23
Is	±0.31	±1.2	23
Waxes	±0.28	±0.9	17

Asphalt. An exception would be if knowledge of the melting points of the wax fraction were important. Then other methods designed only for the determination of waxes should be followed, such as those dependent upon the resin adsorption by clay (16), or the removal of the resins by acid or aluminum chloride (4), or propane (13).

PRECISION. Deviations shown by results of duplicate determinations on 59 asphalts derived from Venezuelan, Mexican, California, Gulf Coast, and various Mid-Continent crudes and refined Trinidad asphalt are given in Table VI. The asphalts ranged from road oils through penetration grades to blown products melting (ring and ball) at 104° C. (220° F.). One cracked road oil was included; other asphalts showed a negative Oliensis spot when tested by the Oliensis unmodified procedure (3). The average deviations were near ±0.3 for each of the five fractions, and for about 80% of the samples the results did not deviate from the average by more than ±0.50 gram per 100 grams of samples. The maximum deviation for all fractions was, on an average, ±1.1 grams per 100 grams of sample. Thus, on two determinations on a sample the average is likely to be close to within ±0.50 gram and with three determinations the average will probably be within ±0.3 gram per 100 grams of sample.

COMPARISON OF WAX CONTENTS. The methylene chloride-acetone dewaxing solvent was found to precipitate contents of waxes intermediate between those obtained by the Holde (9, cf. 1) and the U.O.P. (16) methods, as shown by data of Table VII.

The waxes by the Holde method were essentially free of non-paraffinic material. The drastic heat treatment received during the process apparently converted all resinous materials into lighter oils soluble in the dewaxing solvent. That some of the paraffins are also cracked in the process is shown by the low yield obtained of a low melting point wax.

The U.O.P. method, which depends on clay adsorption to remove resinous material that otherwise might be coprecipitated with the wax, yielded very high contents of waxes from all the asphalts. This method is thus suitable for determining what might be termed the "total content" of waxes, knowledge of which should be of value in interpreting the low-temperature behavior of asphalts. The inclusion of the paraffinic oil with the higher melting waxes did not greatly lower the melting point. This was also noticed in dewaxing trials at various ratios with petrolatums I and II, Table II. The contamination of the waxes with resins apparently has a much greater effect on reduction of melting point. This is probably the reason why the melting point of the wax precipitated by the methylene chloride-acetone solvent from the oxidized flux is lower than that of the wax recovered from the same asphalt by the U.O.P. method, as shown by the difference in specific dispersions of the waxes.

AVERAGE RESULTS OF ANALYSES. Tables VIII and IX list the ranges and average weights of fractions and the properties of fractions separated from blown and straight-run asphalts. The 37 oxidized asphalts were blown chiefly from fluxes reduced from Mid-Continent crudes and blends of these fluxes, but included one California and one Venezuelan oxidized asphalt. The seven 85/100 penetration straight-run asphalts, all of which showed a negative Oliensis spot test (3) in standard naphtha, were

Table VII. Comparison of Wax Contents of Four Asphalts Recovered by Three Methods

Asphalt	Methylene Chloride and Acetone at 0° C. (32° F.)				Holde				U.O.P. Method No. A-46-40			
	Wax found %	Melting point		Specific dispersion × 10 <sup>4</sup>	Wax found %	Melting point		Specific dispersion × 10 <sup>4</sup>	Wax found %	Melting point		Specific dispersion × 10 <sup>4</sup>
		° C.	° F.			° C.	° F.			° C.	° F.	
Mid-Continent flux 1	5.1	59.4	139	125	1.8	51.7	125	95	17.8	53.9	129	115
Mid-Continent flux 2	5.9	50.0	122	113	2.5	47.8	118	100	26.0	46.7	116	115
Oxidized paraffinic-base flux	11.3	62.2	144	126	2.9	52.2	126	98	22.9	65.6	150	108
Paving asphalt	2.3	82+	180+	106	1.5	51.1	124	98	8.7	61.1	142	115



Table VIII. Averages and Ranges of Fractions in Asphalts

	37 Asphalts Oxidized from Bases Reduced from Various Crudes			7 Straight-Run Asphalts Reduced from Different Crudes		
	Minimum	Maximum	Av.	Minimum	Maximum	Av.
S.P. (R. and B.), ° C. (° F.)	69 (157)	104 (220)	93 (200)	43 (110)	48 (119)	46 (115)
Penetration at 25° C. (77° F.)	9	47	25	85	98	92
Hexane-insolubles, %	23	36	32	4	20	15
Hard resins, %	2	33	16	11	39	26
Soft resins, %	13	28	22	22	30	26
Oils, %	10	45	26	14	54	29
Waxes, %	1.0	11.3	4.6	1.4	7.3	3.4

Table IX. Averages and Ranges of Properties of Fractions in Asphalts

Fraction	Hard Resins			Soft Resins			Oils			Waxes		
	Min.	Max.	Av.	Min.	Max.	Av.	Min.	Max.	Av.	Min.	Max.	Av.
37 Asphalts Oxidized from Bases Reduced from Various Crudes												
Density at 65° C., g./ml.	0.946	1.013	0.993	0.910	0.982	0.937	0.898	0.929	0.911	0.853	0.890	0.875
Refractive index <sup>a</sup> at 65° C. (n <sub>D</sub> <sup>65</sup> )	1.56	1.59	1.57	1.51	1.55	1.53	1.503	1.535	1.512	1.472	1.498	1.483
Specific dispersion × 10 <sup>4a</sup>	220	290	240	160	240	190	141	199	156	114	142	130
Refractivity intercept <sup>a</sup>	1.07	1.10	1.08	1.05	1.07	1.06	1.035	1.070	1.056	1.026	1.055	1.047
S.U. viscosity at 98.9° C. (210° F.)	..	..	..	..	..	..	131	1110	215	47	82+	..
M.P. of wax, ° C.	..	..	..	..	..	..	..	..	..	..	..	..
7 Straight-Run Asphalts Reduced from Different Crudes <sup>b</sup>												
Density at 65° C., g./ml.	0.987	1.030	1.012	0.946	0.995	0.962	0.920	0.948	0.937	..	..	..
Refractive index <sup>a</sup> at 65° C. (n <sub>D</sub> <sup>65</sup> )	1.58	1.63	1.60	1.54	1.58	1.55	1.525	1.544	1.530	..	..	..
Specific dispersion × 10 <sup>4a</sup>	230	330	260	200	260	220	159	210	177	..	..	..
Refractivity intercept <sup>a</sup>	1.080	1.115	1.091	1.063	1.082	1.069	1.056	1.070	1.061	..	..	..
S.U. viscosity at 98.9° C. (210° F.)	..	..	..	..	..	..	540	1462	831	..	..	..

<sup>a</sup> Precision of determination on hard and soft resins was low because of difficulty of reading Abbe refractometer.  
<sup>b</sup> Wax contents were low and characterizations were run on only two samples. Therefore, averages and ranges cannot be given.

reduced from two Arkansas, one Venezuelan, two Mid-Continent, one Texas, and one California crude.

The wide range in content of fractions indicates that the method of analysis is highly sensitive to difference in composition of asphalts. With methods based upon adsorption of resins by clay the variation in content and the total content would be less, since the selection is dependent more upon polarity, while isobutyl alcohol, like propane, precipitates fractions more on the basis of molecular weight. An advantage in precipitating relatively large fractions is the greater accuracy of observing changes in composition of asphalts with processing or with service.

The average and range in properties of the fractions obtained upon analysis are shown in Table IX. A wide range in the

character of the components was found, would be expected of fractions precipitated on the basis of molecular weight. While ranges overlap for the different fractions, averages are widespread.

APPLICATIONS. The need to interpret more fully the changes occurring in asphalts under service conditions and during refinery processing led to development of the method of fractionation described herein. Then comparisons could be adjusted in accordance with empirical relations to behavior, by blending various available residues or extracts or by change in refinery processing to make available stock relatively rich in needed fractions. Results of some analyses are given below to illustrate a few of the possible applications.

Steam and vacuum distillation of an asphalt from a penetration

190 to 36 produced changes in fractions as shown by Table X. The softening point (ring and ball) increased from 38.9 to 54.4° C. (102° to 130° F.) to maintain the penetration temperature susceptibility (15) very nearly constant. As expected, the first overhead products were largely oils, but to reach lower penetrations loss of soft resins also occurred. The aromaticity of the fractions remaining in the asphalt did not change greatly, as is shown by the nearly constant values for specific dispersion. The oil fraction became much more viscous.

Air blowing of the straight-reduced asphalt of 36 penetration markedly increased the content of hexane-insolubles at the expense of the resins, especially the hard resins. These changes in the fractional composition, which are graphed on Figure 2, softened point for both the distillation and blowing operation served to decrease the penetration and to raise the softening

Table X. Analyses of Asphalts

Properties of asphalts	Straight-Reduced			Air-Blown from Asphalt of 36 Penetration		
	190	89	36	13	9	1
Penetration at 25° C. (77° F.)	190	89	36	13	9	1
Softening point (R. and B.), ° C. (° F.)	38.9 (102)	45.0 (113)	54.4 (130)	85.0 (185)	96.1 (205)	173.3 (344)
PTS × 100 <sup>a</sup>	4.5	4.7	4.6	3.0	2.7	0.2
Fractions, % by wt.						
Hexane-insolubles	11.4	12.3	14.8	26.9	31.4	51.3
Hard resins	39.5	41.2	45.5	36.6	36.1	19.6
Soft resins	26.8	27.8	25.0	22.3	20.9	16.9
Oils	20.4	15.8	12.3	11.9	10.0	11.1
Waxes	2.1	2.9	2.5	2.0	1.8	1.6
Total	100.2	100.0	100.1	99.7	100.2	100.5
Ratio of total resins to hexane-insolubles	5.8	5.6	4.7	2.2	1.8	0.7
Specific dispersion of fractions <sup>b</sup> × 10 <sup>4</sup>						
Hard resins	235	230	235	216	231	232
Soft resins	193	194	188	189	189	172
Oils	166	173	166	158	168	154
Viscosity of oils, S.U.S. at 98.9° C. (210° F.)	446	862	932	949	993	644

<sup>a</sup> PTS =  $\frac{\log 800 - \log \text{pen. at } 25^\circ \text{C.}}{\text{S.P. (R. and B.) } ^\circ \text{C.} - 25}$   
<sup>b</sup> Calculated from values of density and of refractive index as determined with an Abbe refractometer. Determinations of refractive index were made on resins at 65° C. (n<sub>D</sub><sup>65</sup>) and on oils at 25° C. (n<sub>D</sub><sup>25</sup>). Determinations of density were made on resins at 65° C. and on oils at 25° C.

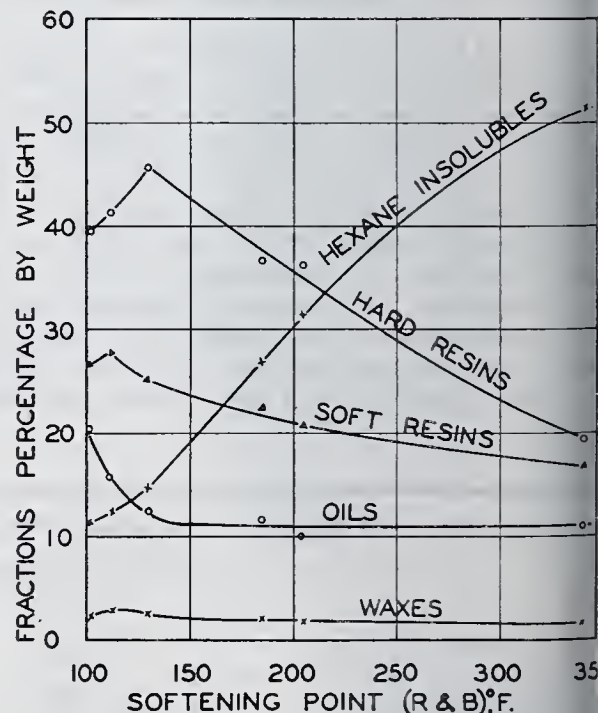


Figure 2. Change in Fractions during Distillation and Air-Blowing of Asphalts



Table XI. Analyses of Asphalts

Air-blown from bases of varying consistency straight-reduced from smackerover crude oil and analysis of smackerover asphalt)						
	Blown Asphalts					Straight-Reduced
Properties of base						
Penetration at 50° C. (122° F.), seconds	78	83	140	530	740	750
Softening point at 25° C. (77° F.)	...	...	...	110	91	89
Softening point (R. and B.), ° C. (° F.)	...	...	...	44.4 (112)	46.1 (115)	45.0 (113)
Properties of blown asphalt						
Penetration at 25° C. (77° F.)	28	34	23	15	18	89
Softening point (R. and B.), ° C. (° F.)	96.1 (205)	83.3 (182)	95.0 (203)	94.4 (202)	83.3 (182)	45.0 (113)
TS × 100	2.0	2.3	2.2	2.5	2.8	4.7
Fractions in blown asphalt, % by wt.						
Hexane insolubles	33.6	31.2	32.3	33.1	29.4	14.2
Hard resins	10.1	12.8	22.3	32.8	30.9	42.5
Soft resins	22.6	23.8	21.6	20.3	25.9	25.9
Oils	31.9	29.2	21.8	11.5	11.5	14.3
Waxes	3.2	3.3	2.8	2.9	3.2	3.2
Total	101.4	100.3	100.8	100.6	100.9	100.1
Ratio of total resins to hexane insolubles	1.0	1.2	1.4	1.6	1.9	4.8
Specific dispersion of fractions × 10 <sup>4</sup>						
Hard resins	245	241	242	231	237	234
Soft resins	188	195	188	191	183	188
Oils	158	151	159	169	161	166
Viscosity of oils, S.U.S. at 98.9° C. (210° F.)	171	205	262	366	621	701

Calculated from values of density and of refractive index as determined with an Abbe refractometer. Determinations of refractive index were made on resins at 65° C. ( $n_D^{65}$ ) and on oils at 25° C. ( $n_D^{25}$ ). Determinations of density were made on resins at 65° C. and on oils at 25° C.

Table XII. Analyses of Solvent Extracts and Propane Precipitates

Stock	Agent	Viscosity of Extracts and Precipitates S.U.S. at 98.9° C. (210° F.)	Fractions Found, % by Wt.						Specific Dispersion × 10 <sup>4</sup> of Fractions <sup>a</sup>			Viscosity of Oil Fractions S.U.S. at 98.9° C. (210° F.)
			H.I.	H.R.	S.R.	O.	W.	Total	H.R.	S.R.	O.	
Crude	Phenol	780	0.0	0.0	10.9	89.6	0.2	100.7	...	214	196	558
Crude	Furfural	1770	0.2	1.1	24.1	73.4	1.3	100.1	...	243	242	899
Crude	Chloroform	1093	0.8	0.0	9.3	85.6	3.5	99.2	...	160	162	116
Crude	Propane	567 (Saybolt Furol at 210° F.)	0.0	71.5	16.2	9.9	3.3	100.9	242	216	156	501
Crude	Propane	916	0.0	56.9	25.7	14.8	3.3	100.7	...	149	153	412
Crude	Propane	b	35.0	50.0	9.1	5.0	1.2	100.3	290	275	213	...

Calculated from values of density and of refractive index as determined with an Abbe refractometer. Determinations of refractive index were made on resins at 65° C. ( $n_D^{65}$ ) and on oils at 25° C. ( $n_D^{25}$ ). Determinations of density were made on resins at 65° C. and on oils at 25° C. Softening point (R. and B.), 103° C. (218° F.). Penetration at 25° C. (77° F.), 1.

of the asphalt, and therefore to lower greatly the penetration-temperature susceptibility. This decrease was found to be related to the ratio of the total resin to the hexane-insoluble content, as is shown by curve 1 of Figure 3. The character of the fractions obtained from the air-blown asphalts was remarkably constant, as shown by measurements of specific dispersion and viscosity of the oils, until a softening point above 205° C. (205° F.) had been reached. Thereafter, the softening point and oil fractions from the asphalt became less aromatic and the oil fraction was lowered in viscosity, as shown by the data in Table XII. In the manufacture of blown asphalts it is necessary in many instances to obtain products of approximately the same softening point but of varying penetration. The common practice, with values available from a given crude oil, is to air-blow bases of varying viscosity. In general, the higher the viscosity of the base the lower will be the penetration of the blown asphalt at a given melting point. The fractional compositions of a series of blown asphalts, all melting within the range of 82.2° to 96.1° C. (205° to 205° F.), but prepared from bases of varying consistency are shown by Table XI. A nearly constant content of hexane-insolubles was found in this series. The result of blowing an asphalt base of lower oil content but with an essentially constant ratio of resins to insolubles is thus to obtain an end product of given melting point but with a higher ratio of resins to insolubles, since less conversion of resins to insolubles has occurred. The ratio of total resins to hexane-insolubles for the blown asphalts and for a straight-run asphalt of 87 penetration

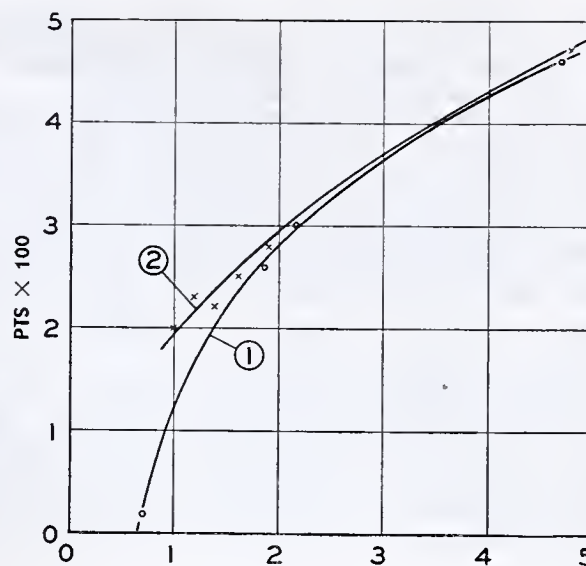


Figure 3. Penetration-Temperature Susceptibility vs. Ratio of Total Resins to Hexane-Insolubles

from the same crude, analysis of which is also shown in Table XI, was again found related to penetration-temperature susceptibility (curve 2, Figure 3). Values for penetration-temperature susceptibility are at a slightly higher level than shown by data on the blown asphalts of Table X, since the asphaltic crude bases involved, although similar, were different.

The fractional compositions of three solvent extracts and of three propane precipitates are shown in Table XII. Although no direct comparison may be made of the action of the different refining agents because of the difference in starting stocks, the precipitating action of propane as compared with extracting effect of the selective solvents is readily apparent. Such analyses indicate the value of extracts and precipitates in blending procedures for adjusting compositions of petroleum residua. Thus, for example, the propane precipitates contain large contents of resins and could therefore be added to residua to raise their penetration-temperature susceptibility and increase ductility;

Table XIII. Analyses of Road Oil

Crude Base	Paraffinic	Paraffinic	Asphaltic
Properties of road oil			
Viscosity, S. Furol at 60° C. (140° F.), seconds	440	640	700
Flash point, Cleveland open cup, ° C. (° F.)	316 (600)	210 (410)	154 (310)
Spot test	Neg.	Pos.	Neg.
Specific gravity at 25° C./25° C. (77° F./77° F.)	0.963	1.034	0.990
Fractions, % by wt.			
Hexane insolubles	2.5	12.2	18.9
Hard resins	19.4	2.6	12.1
Soft resins	38.7	16.6	11.6
Oils	31.2	58.8	54.5
Waxes	8.2	5.9	1.1
Loss of oils by evaporation	0.0	3.9	1.8
Total	100.0	100.0	100.0
Specific dispersion of fractions × 10 <sup>4</sup>			
Hard resins	303	...	...
Soft resins	204	238	...
Oils	162	247	150
Refractive index of hard resin, $n_D^{65}$ , ° C.	1.577	1.623	1.589
Viscosity of oils, S.U.S. at 98.9° C. (210° F.)	261	139	106

<sup>a</sup> Calculated from values of density and of refractive index as determined with an Abbe refractometer. Determinations of refractive index were made on resins at 65° C. ( $n_D^{65}$ ) and on oils at 25° C. ( $n_D^{25}$ ). Determinations of density were made on resins at 65° C. and on oils at 25° C.



while the extracted aromatic oils of high viscosity could be substituted for paraffinic-type oils removable by distillation or solvent treatment, thereby to improve the homogeneity of asphalts, as might be desirable in certain applications.

The differences found in content and character of fractions in three SC-6 road oils (Table XIII), illustrates the wide variety of such products available. The highly paraffinic Mid-Continent road oil derives its viscosity mainly from highly viscous resin and oil fractions instead of a normal content of hexane-insolubles. For this reason it would be slow to set upon the road and would form a brown-colored bituminous mat likely to "bleed". The asphaltic-type road oil, while having a very high content of insolubles, has always rated highly in service performance.

The analysis of an oil formed by mild cracking of a paraffinic Mid-Continent stock is shown in column 2, Table XIII. This oil is low in hard resin and high in wax content. The high hexane-insoluble and wax content and low content of hard resins indicate that after a period of exposure this road oil would become nonhomogeneous and deterioration of the road mat would result for this reason.

#### SUMMARY

The procedure of analysis has been found adequate for accurate classification of asphalts from widely differing sources according to the content and character of their five main fractions. The method is suitable for products varying in consistency from road oils to highly blown products. While only a few trials have been made on cracked asphalts, the treatment is thought also to be applicable in the separation of their fractions, except for some products produced by high level cracking which, although resinous, are largely insoluble in hexane. Other petroleum products which contain some or all of the fractions found in residua may also be subjected to analysis, such as extracts and precipitates from lubricating stocks and lubricating oils which have been in heavy-duty service.

#### ACKNOWLEDGMENT

The authors wish to express their appreciation to John W. Poole for many helpful suggestions and to Janet M. Lemley for assistance in the laboratory determinations.

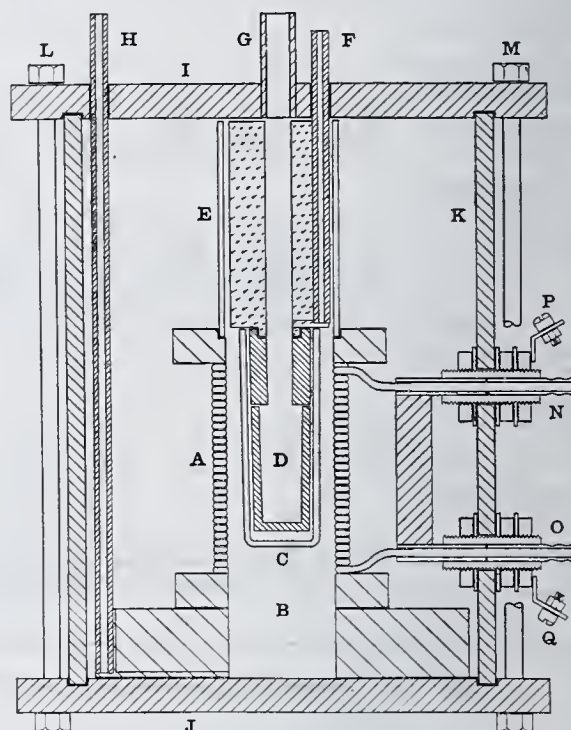
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## A Controlled-Atmosphere Induction Melting Furnace for the Laboratory

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THE most common controlled-atmosphere (or vacuum) furnace for melting small metal samples by induction has been described frequently and is supplied as standard equipment by the Ajax Electrothermic Corporation. Its construction entails the use of silica tubing which is fragile, expensive and difficult to work without special glass-blowing equipment. The disadvantages in the use of silica have led to the development in this laboratory of a less delicate piece of equipment which serves the same purpose and can be constructed in a few hours in a well-equipped shop.

Important construction features are shown in the diagrammatic drawing. In essence, the heating unit is merely enclosed by gas-tight shell so that the entire assembly, including the furnace coil, can be evacuated or flushed with inert gas.

The water-cooled heating coil, A, is connected with the converter cables at P and Q and with water lines at N and O. It is the standard coil supplied with the 3 kv.-amp. furnace assembly. The Alumundum thimble, C, contains the melting crucible, D, and is surrounded with Norconite, B, or other suitable insulating powder. A layer of sheet mica, rolled into a cylinder separates this powder from the cooling coil, and prevents its lateral escape. The assembly, E, above the melting crucible serves to diminish the upward radiation from the molten charge while permitting a clear view of the charge through sight tube G.

This whole unit is surrounded by the chamber formed by fiber plates, I and J, and leucite (or metal) cylinder, K. Rubber gaskets in the grooves of the top and bottom plate and at the water-cooled electrical connections render the entire assembly gas-tight when compressed by the six vertical bolts, L, M, etc. and the brass nuts and washers of the cooling tubes. The gas input and exit tubes, H and F, are sealed with DeKhotinsky cement to the top plate, as is also G. The temperature of the molten charge may be determined either by an optical pyrometer, sighted through a cemented window at G, or by a thermocouple, within a gas-tight protection tube, inserted into the charge through G and cemented at the top. Insulation is unnecessary outside the water-cooled coil and the bolts are far enough from the induction currents to remain cool, despite crucible temperatures of 1500° C. or above. The crucible and contents cool rapidly when the current is interrupted and may be replaced in a few minutes by removal of the top plate and the radiation shields.

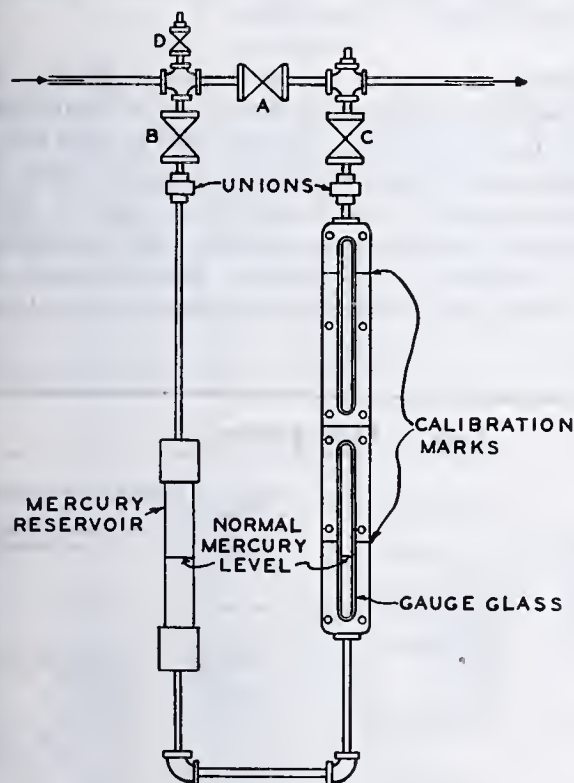
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# Positive Displacement Flowmeter

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LABORATORY and pilot-plant work, particularly when continuous processes are studied, it is often necessary to measure the rate of flow of small, sometimes pulsating or irregularly flowing streams which may vary in density or viscosity. In many such instances it is inconvenient or impossible to use conventional orifice- or Venturi-type meters, rotameters, or graduated tanks. Obviously, some form of positive displacement meter is desirable for such service; but unfortunately most commercially available meters of this type are either too large, have pressure, temperature, or other limitations which make them unsuitable.



A problem often encountered in these laboratories which is thus complicated is the measurement of recycle or "combined feed" streams in continuous cracking pilot plants. Often these streams are hot and are subject to changes in temperature and composition in the course of a run. Usually they are transferred by a reciprocating pump, and always the continuity of their flow must be maintained. While alternately used surge tanks, pump calculations, and calibrated orifices or rotameters can be employed with some success, all these methods have certain drawbacks in such use. The device described below (1) was developed especially for this service and has been in use about 2.5 years. It is also applicable to many other flow-measurement problems, and has been found particularly useful in obtaining rapid, approximate measurements of flow rates even when the quantity flowing over a long period is determined by more accurate means, such as calibrated charge tanks. Its chief advantages are simplicity of construction and operation; applicability over wide ranges of temperature, pressure, and stream composition; and a positive calibration which does not change under operating conditions or fluid properties.

The diagram shows the particular design of this flowmeter which has been most generally used in the author's laboratories. It consists essentially of a U-tube, one leg of which is a glass, the other a reservoir for mercury, water, or other liquid heavier than and immiscible with the flowing stream. Ferguson "Reflex" type glass has been used most commonly because it allows operation at high temperatures and pressures, but any glass satisfactory for the prevailing condi-

tions may be used. The mercury reservoir can be a suitable length of pipe or any convenient vessel. The U-tube is connected by means of unions to valves B and C, which in turn join the line carrying the stream to be measured. Valve A is interposed between the point of connection of B and C. The crosses and plugs shown and valve D are provided for convenience in filling and cleaning.

Before the gage glass is connected, its volume between two convenient markings is determined. When the device is in operation the flow is normally in the direction shown in the sketch, with A, B, and C open. To measure the flow rate, A is closed, forcing the oil (or other fluid being measured) into the mercury reservoir, the mercury into the gage glass, and oil out of the gage glass into the main line. Since as much oil is forced out of the system as enters, the net rate of flow in the main line remains unchanged. The measurement itself consists in determining the time required for the mercury level to rise from one calibration mark to the other, thus filling a known volume. When this measurement is completed, A is reopened, and the mercury seeks its own level. Here again, since oil is discharged from one leg as rapidly as it enters the other, no interruption in the net flow occurs.

Calculation of the flow rate from the observed time is simple. Thus, if the calibrated volume is  $V$  gallons, and the time required to fill it is  $t$  minutes, the rate of flow,  $R$ , in gallons per minute, is obtained by the equation  $R = \frac{V}{t}$ . In one typical example, the calibrated volume was 0.046 gallon and the observed filling time was 30 seconds. The rate of flow was then  $\frac{0.046}{30} \times 60 = 0.092$  gallon per minute.

While the design described is cheap, simple, and highly satisfactory, it can be modified to meet particular demands and utilize existing apparatus. The calibrated volume should be of such a size that the time required for a single determination is neither so long as to be inconvenient nor so short as to be inaccurate. The author has found 30 to 60 seconds satisfactory.

More elaborate modifications of the basic design have been suggested (1) for applications where an automatically determined rate or a record of total flow is desired. While the device was developed for, and to date has been limited to, the measurement of liquid streams varying from liquid propane to heavy recycle oil, it can also be used for measuring gas streams by taking proper account of the compression of the gas on the upstream side of the meter, using a low-density sealing liquid, and limiting the variation in liquid level to a low value.

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THIS device was described by L. S. Kassel of the U.O.P. Research and Development Laboratories at the round table discussion on pilot-plant design, construction, and operation, Division of Petroleum Chemistry, 106th Meeting of the AMERICAN CHEMICAL SOCIETY, Pittsburgh, Pa.

## Course in Instrumental Methods of Analysis and Control

An ESMWT course in instrumental methods of analysis and control will be given at the University of Southern California, Los Angeles, starting June 12. The course now being taught by Sidney W. Benson is to be expanded for the summer session to 7 hours of laboratory work and one hour of lecture each week for 15 weeks, devoted to study of the applications of physical properties of chemical substances to the analysis and control of chemical processes, and including laboratory work with basic instruments such as pressure gages, flowmeters, rotameters, thermocouples, potentiometric circuits, colorimeters, spectrophotometers, and electronic relays.



# Phenyl Isocyanate Derivatives of Certain Alkylated Phenols

## Melting Points and X-Ray Powder Diffraction Data

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As positive means for identifying alkylated phenols, the authors have prepared phenyl isocyanate derivatives of a large number of phenols, and in this paper present tables of melting points and x-ray powder diffraction data.

AS A result of recent developments, considerable quantities of alkylated phenols are finding use in the preparation of synthetic plastics, rubber, germicides, fungicides, and related substances. Because of this increasing interest in alkylated phenols, the authors have undertaken to develop an easy and positive means for their identification. One widely used method for such purposes is the determination of the characteristic melting point of the aryl *N*-phenylcarbamate formed by the reaction of a phenol with phenyl isocyanate. As this procedure has been shown to be satisfactory, it was decided to prepare these derivatives of a large number of phenols which were available, and then to ascertain their melting points and also to obtain their x-ray powder diffraction data. Either the melting point or the x-ray powder diffraction pattern of the phenyl isocyanate derivative is usually sufficient for identification of the parent phenol, but a consideration of both determinations gives results which are unequivocal.

The melting points of a number of such phenolic derivatives have been listed by Morgan and Pettet (14) and by Steinkopf and Höpner (16); a few relevant supplemental data are given by other investigators. No previous publications have been found in which x-ray powder diffraction data are presented for these derivatives, although the value of such information for the identification of compounds possessing crystalline structure has been pointed out by Hanawalt and co-workers (7, 8), Davey (3), and others.

### PROCEDURES

The method of preparation of the aryl *N*-phenylcarbamates was based upon the procedures given by Steinkopf and Höpner (16) and Weehuizen (21). A small quantity of the phenolic substance (ca. 1 gram) was mixed with a slight molar excess of phenyl isocyanate in a 20-cm. (8-inch) test tube fitted with a reflux condenser and to this reaction mixture were added 8 to 10 ml. of a petroleum distillate (b.p. 170–200° C.) previously fractionated from kerosene. The reaction was completed by gently refluxing for 1 to 4 hours (usually 4). After product was cooled, the crystals which formed were recovered by filtration and purified by recrystallization from petroleum ether, benzene, or a mixture of these solvents. The purified derivative was dried under room conditions and its melting point was determined using a calibrated Anschütz thermometer.

The x-ray diffraction patterns of the aryl *N*-phenylcarbamates were secured by the usual Debye-Scherrer-Hull method. A small portion of the material was finely powdered, packed into a short length of 19-gage stainless steel hypodermic needle tubing of 0.7-mm. internal diameter, compressed with a plunger, and finally extruded as a cylinder of the same diameter. The steel tubing was mounted in a camera of 57.3-mm. effective diameter in such a manner that only the extruded part of the specimen appeared in the x-ray beam. The photographs were made with filtered  $\text{CuK}\alpha$  radiation having an effective

wave length of 1.539 Å. A camera of 171.9-mm. effective diameter was employed to obtain better resolution of the diffraction patterns of some of the samples.

The diffraction patterns obtained were, in general, sharp defined and the measurement of them could be duplicated satisfactorily. In a few instances, however, two or more diffraction lines representing different interplanar spacings merged, making exact interpretation of a specific portion of a pattern uncertain. When this was the case, the merged lines were considered as a group and measured as a single line. The interplanar spacing calculated from such a measurement represents the shortest inter-

Table I. Melting Points

X-Ray Diffraction Pattern No.	Empirical formula	Phenols Used Name	M.P. of <i>N</i> -Phenylcarbamates, ° Observed by authors	Literature values references
1	$\text{C}_6\text{H}_5\text{ClO}$	4-Chlorophenol	148.5	.....
2	$\text{C}_6\text{H}_5\text{NO}_3$	2-Nitrophenol (m.p. 44.5° C.)	.....	.....
3	$\text{C}_6\text{H}_5\text{NO}_3$	4-Nitrophenol	156	.....
4	$\text{C}_6\text{H}_5\text{O}$	Phenol	126.5	126 (6, 14), 125.5 (124 (12, 16), 122 (125 (16)
5	$\text{C}_6\text{H}_5\text{S}$	Thiophenol	128.5	125 (16)
6	$\text{C}_7\text{H}_5\text{O}$	2-Methylphenol	142.5	145 (12, 17), 144 (16), 141 (14), (6), 141 (20)
7	$\text{C}_7\text{H}_5\text{O}$	3-Methylphenol	124.5	125-6 (17), 125 (6, 124.5 (16), 121 (20)
8	$\text{C}_7\text{H}_5\text{O}$	4-Methylphenol	113	115 (5, 14), 114 (16, 17), 112-13 (1)
9	$\text{C}_7\text{H}_5\text{S}$	4-Methylthiophenol	132	.....
10	$\text{C}_8\text{H}_{10}\text{O}$	2-Ethylphenol	143.5	141 (16, 19), 140-1 (140 (18)
11	$\text{C}_8\text{H}_{10}\text{O}$	3-Ethylphenol	137 <sup>b</sup>	138.8 (16), 138 (11)
12	$\text{C}_8\text{H}_{10}\text{O}$	4-Ethylphenol	120.5	120 (11, 16, 19)
13	$\text{C}_8\text{H}_{10}\text{O}$	2,3-Dimethylphenol	173.5	176 (16)
14	$\text{C}_8\text{H}_{10}\text{O}$	2,4-Dimethylphenol	103 <sup>c</sup>	112 (14, 16), 111 (122.2 (4), 102 (5, 1)
15	$\text{C}_8\text{H}_{10}\text{O}$	2,5-Dimethylphenol	166	162 (5, 14, 16), 160 (1)
16	$\text{C}_8\text{H}_{10}\text{O}$	3,4-Dimethylphenol	119.3	120 (14, 16)
17	$\text{C}_8\text{H}_{10}\text{O}$	3,5-Dimethylphenol	149.5	151 (5, 16), 148 (14)
18	$\text{C}_9\text{H}_{12}\text{O}$	2,4,6-Trimethylphenol	143	142 (16), 141-2 (140-2 (1)
19	$\text{C}_{10}\text{H}_{13}\text{ClO}$	2- <i>tert</i> -Butyl-4-chlorophenol	133	.....
20	$\text{C}_{10}\text{H}_{14}\text{O}$	4- <i>tert</i> -Butylphenol	148.5	.....
21	$\text{C}_{11}\text{H}_{14}\text{O}$	4-Methyl-2-( $\beta$ -methylallyl)phenol	98.5	.....
22	$\text{C}_{11}\text{H}_{16}\text{O}$	4- <i>tert</i> -Amylphenol	108	.....
23	$\text{C}_{11}\text{H}_{16}\text{O}$	4(or 6)- <i>tert</i> -Butyl-2-methylphenol (b.p. 135° C. at 20 mm.)	139.5	.....
24	$\text{C}_{11}\text{H}_{16}\text{O}$	6(or 4)- <i>tert</i> -Butyl-2-methylphenol (b.p. 123° C. at 20 mm.)	189	.....
25	$\text{C}_{11}\text{H}_{16}\text{O}$	4(or 6)- <i>tert</i> -Butyl-3-methylphenol (b.p. 129° C. at 20 mm.)	133	.....
26	$\text{C}_{11}\text{H}_{16}\text{O}$	2- <i>tert</i> -Butyl-4-methylphenol	155	.....
27	$\text{C}_{12}\text{H}_{18}\text{O}$	4-Phenylphenol	167.5	.....
28	$\text{C}_{12}\text{H}_{14}\text{O}_3$	2,6-Diacetyl-3,5-dimethylphenol (m.p. 109° C.)	.....	.....

<sup>a</sup> These phenols did not form phenyl isocyanate derivatives.

<sup>b</sup> This value was had only after thorough drying of the sample. Without drying a value of 124° C. was obtained consistently.

<sup>c</sup> This value was found consistently even after several recrystallizations and drying.



interplanar spacing of the group considered and is followed by the letter D in Table II.

Certain flaky organic crystals tend to pack anisotropically, which results in preferred orientation of the crystallites in the extruded specimen when prepared in the manner employed in this work. Since it is necessary that the powdered specimen employed in a Debye-Scherrer-Hull diffraction photogram contain crystallites randomly oriented, diffraction data were secured for a number of the compounds studied, using a rotating powdered sample loosely packed in a thin-walled nylon tube in order to determine whether anisotropic packing of the extruded specimens occurred. Patterns obtained with loosely packed specimens checked those secured with extruded specimens, and inasmuch as all the compounds studied were similar, these data were taken to indicate that there was no preferred orientation of crystallites in the extruded specimens examined.

# DISCUSSION

The phenols studied were mainly of the alkylated type, where alkyl groups were methyl, ethyl, isopropyl, *tert*-butyl, and *n*-amyl, although a few contained other substituents. In Table I set forth a complete list of these phenols along with the melting points of their phenyl isocyanate derivatives. In the first column of this table are also listed the diffraction pattern numbers for cross reference to Table II, which contains the x-ray diffraction data of the phenyl isocyanate derivatives.

In certain cases no reaction between the phenol and phenyl isocyanate occurred even after as much as 15 hours of refluxing. In

Table I. Melting Points (Cont'd)

X-ray Diffraction Pattern No.	Empirical formula	Phenols Used Name	M.P. of N-Phenylcarbamates, ° C.	
			Observed by authors	Literature values and references
29	C <sub>12</sub> H <sub>16</sub> O	2-Cyclohexylphenol	111.5	.....
30	C <sub>12</sub> H <sub>16</sub> O	4-Cyclohexylphenol	145.5	.....
31	C <sub>12</sub> H <sub>16</sub> O	2- <i>tert</i> -Amyl-4-methyl-phenol	124	.....
32	C <sub>12</sub> H <sub>18</sub> O	4(or 6)- <i>tert</i> -Butyl-3-ethylphenol (b.p. 142° C. at 20 mm.)	156	.....
33	C <sub>12</sub> H <sub>18</sub> O	2- <i>tert</i> -Butyl-4-ethylphenol	134	.....
34	C <sub>12</sub> H <sub>18</sub> O	4(or 6)- <i>tert</i> -Butyl-2,3-dimethylphenol (b.p. 139° C. at 20 mm.)	216	.....
35	C <sub>12</sub> H <sub>18</sub> O	6- <i>tert</i> -Butyl-2,4-dimethylphenol	173	.....
36	C <sub>12</sub> H <sub>18</sub> O	4- <i>tert</i> -Butyl-2,5-dimethylphenol	144	.....
37	C <sub>12</sub> H <sub>18</sub> O	4- <i>tert</i> -Butyl-2,6-dimethylphenol	160	.....
38	C <sub>12</sub> H <sub>18</sub> O	6- <i>tert</i> -Butyl-3,4-dimethylphenol	142	.....
39	C <sub>12</sub> H <sub>18</sub> O	2,6-Diethyl-3,5-dimethylphenol	226	.....
40	C <sub>13</sub> H <sub>20</sub> O	3,5-Diisopropyl-2-methylphenol	198.5	.....
41	C <sub>13</sub> H <sub>20</sub> O	3,5-Diisopropyl-4-methylphenol	256	.....
42	C <sub>14</sub> H <sub>22</sub> ClO	2,6-Di- <i>tert</i> -butyl-4-chlorophenol (m.p. 78° C.)	<sup>a</sup>	.....
43	C <sub>16</sub> H <sub>24</sub> O	4,6-Di- <i>tert</i> -butyl-2-methylphenol	163.5	.....
44	C <sub>16</sub> H <sub>24</sub> O	4,6-Di- <i>tert</i> -butyl-3-methylphenol	171.5	.....
45	C <sub>16</sub> H <sub>24</sub> O	2,6-Di- <i>tert</i> -butyl-4-methylphenol (m.p. 69.5° C.)	<sup>a</sup>	.....
46	C <sub>16</sub> H <sub>24</sub> O	2- <i>tert</i> -Butyl-4-cyclohexylphenol	170	.....
47	C <sub>16</sub> H <sub>26</sub> O	4,6-Di- <i>tert</i> -butyl-3-ethylphenol	182.5	.....
48	C <sub>16</sub> H <sub>26</sub> O	2,6-Di- <i>tert</i> -butyl-4-ethylphenol (m.p. 43.5° C.)	<sup>a</sup>	.....
49	C <sub>16</sub> H <sub>26</sub> O	4,6-Di- <i>tert</i> -butyl-2,3-dimethylphenol	216	.....
50	C <sub>18</sub> H <sub>30</sub> O	2,4,6-Tri- <i>tert</i> -butylphenol (m.p. 131° C.)	<sup>a</sup>	.....
51	C <sub>20</sub> H <sub>32</sub> O	2,6-Di- <i>tert</i> -butyl-4-cyclohexylphenol (m.p. 115.5° C.)	<sup>a</sup>	.....

<sup>a</sup> These phenols did not form phenyl isocyanate derivatives.

Table II. Powder Diffraction Data<sup>a</sup>

<i>d</i>	<i>I</i> / <i>I</i> <sub>1</sub>	<i>d</i>	<i>I</i> / <i>I</i> <sub>1</sub>	<i>d</i>	<i>I</i> / <i>I</i> <sub>1</sub>
1b. 4-Chlorophenyl N-phenylcarbamate		2b. 2-Nitrophenol		3b. 4-Nitrophenyl N-phenylcarbamate	
5.9	W	7.5	(3) S	21.0D	W
5.4	W	6.2	VW	10.4D	W
5.9	(1) VS	5.9	(2) S	6.3	VW
4.61	W	5.0	M	5.9	W
4.41	W	4.86	W	5.3	VW
4.28	W	3.87	M	4.94	W
4.16	W	3.65	W	4.63D	(1) VS
4.05	W	3.25D	(1) VS	4.14	M
3.89	M	3.10	W	4.03	M
3.71	(2) S	3.01	W	3.82	(2) VS
3.62	VW	2.92	W	3.58	(3) VS
3.48	VW	2.76	VW	3.47	W
3.18	(3) S	2.64	W	3.39	W
3.09	M	2.55	W	3.32	W
2.99	W	2.47	W	3.17	W
2.88	VW	2.43	VW	3.05	W
2.69	W	2.31	VW	3.00	VW
2.30	W	2.26	W	2.83	VW
2.24	VW	2.19	VW	2.70	VW
2.19	VW	2.13	M	2.66	VW
1.90	VW	2.09	VW	2.58	W
1.86	VW	2.02	VW	2.47	VW
1.81	VW	1.96	W	2.34	W
		1.92	VW	2.29	VW
		1.85	W	2.24	W
		1.82	VW	2.16	VW
		1.75	VW	2.08	W
		1.73	W	2.02	W
		1.64	VW	1.89	W
				1.84	W

4. Phenyl N-phenylcarbamate		5b. S-Phenyl N-phenylthio- carbamate		6. 2-Methylphenyl N-phenylcarbamate	
6.8	M	7.9	(3) S	7.6	S
4.36D	(1) VS	7.0	W	6.1	W
3.98	(2) S	6.7	W	5.2	(1) VS
3.48D	S	6.2	W	4.61	(2) VS
3.21	S	5.5	M	4.20	(3) VS
3.09	W	4.78	(1) VS	3.62D	S
2.96	W	4.56	VW	3.17	S
2.71D	(3) S	4.30	(2) S	2.85	W
2.63	M	4.22	W	2.62	W
2.53	M	4.10	W	2.43	W
2.46	M	4.03	W	2.30	W
2.30	M	3.89	W	2.09	M
2.21	M	3.58	VW	1.99	M
2.12	M	3.33D	M	1.87	VW
1.99	M	3.19	W	1.67	VW
1.90	W	3.01	W	1.60	VW
1.83	M	2.85	W		
1.74	M	2.72	W		
1.66	W	2.61	M		
1.60	W	2.49	VW		
1.53	W	2.41	VW		
1.47	VW	2.28	W		
1.41	VW	2.14	VW		
1.35	VW	2.09	VW		
1.30	VW	2.03	VW		
1.18	VW	1.89	VW		
1.10	VW	1.84	VW		
1.08	VW				

7b. 3-Methylphenyl N-phenylcarbamate		8. 4-Methylphenyl N-phenylcarbamate		9b. S-4-Methylphenyl N-phenylthio- carbamate	
8.2	S	4.66	(1) VS	10.9	S
7.5	VW	4.03	(2) S	10.0	S
6.4	VW	3.67	(3) S	8.3	VW
5.3	(3) S	3.14	M	6.7	VW
5.0	VW	2.96	VW	5.8	M
4.61	(2) S	2.64	W	5.3	VW
4.47	VW	2.33	M	4.86	M
4.22	(1) VS	1.99	VW	4.48	(1) VS
4.03	M	1.89	VW	3.80	M
3.78	W			3.59	(2) S
3.64	W			3.49	S
3.50	W			3.24	VW
3.38	VW			3.08	(3) S
3.29	VW			2.99	VW
3.17	M			2.84	W
3.05	VW			2.57	M
2.89	W			2.52	W
2.81	W			2.42	W
2.68	VW			2.31	W
2.54	W			2.19	W
2.42	W			2.12	W
2.37	W			1.95	VW
2.30	VW			1.92	VW
2.25	VW			1.80	VW
2.19	VW			1.76	VW
2.09	VW				
2.02	VW				
1.96	VW				

<sup>a</sup> *d* = interplanar spacing in Ångströms; *I*/*I*<sub>1</sub> = estimated relative intensity; S = strong; M = medium; W = weak; V = very. Three strongest lines indicated in decreasing order of intensity by (1), (2), and (3). For explanation of D see text.

<sup>b</sup> Data obtained using a camera of 171.9-mm. effective diameter.



Table II. Powder Diffraction Data<sup>a</sup> (Cont'd)

<i>d</i>	<i>I</i> / <i>I</i> <sub>1</sub>	<i>d</i>	<i>I</i> / <i>I</i> <sub>1</sub>	<i>d</i>	<i>I</i> / <i>I</i> <sub>1</sub>	<i>d</i>	<i>I</i> / <i>I</i> <sub>1</sub>	<i>d</i>	<i>I</i> / <i>I</i> <sub>1</sub>	<i>d</i>	<i>I</i> / <i>I</i> <sub>1</sub>
10. 2-Ethylphenyl N-phenylcarbamate		11. 3-Ethylphenyl N-phenylcarbamate		12. 4-Ethylphenyl N-phenylcarbamate		13. 2,3-Dimethyl- phenyl N-phenylcarbamate		14. 2,4-Dimethyl- phenyl N-phenylcarbamate		15 <sup>b</sup> . 2,5-Dimethyl- phenyl N-phenyl- carbamate	
7.9	S	7.9	S	7.3	M	7.8	M	5.6	M	7.7	(2)
6.3	M	6.2	M	6.5	W	6.2	W	5.1	M	7.2	(2)
5.4	(2) VS	5.3	(2) S	5.5	W	5.4	(3) S	4.52	(1) VS	6.6	
4.71	S	4.63	(3) S	4.73	(2) S	4.66	(2) VS	4.30	M	5.6	
4.22	(1) VS	4.18	(1) VS	4.32	(1) VS	4.24	(1) VS	4.05	W	5.0	
4.07	S	4.03	S	3.94	(3) S	3.99	M	3.65	(3) M	4.56	(3)
3.67	(3) S	3.62	S	3.58	M	3.68	S	3.31	(2) S	4.33	(1)
3.20D	S	3.22	S	3.25D	S	3.21D	S	3.02	W	4.05	
2.98	W	2.94	M	2.83	M	3.01	W	2.76	W	3.91	
2.77	M	2.70	M	2.58	W	2.75	W	2.57	W	3.80	
2.59	M	2.61	M	2.45	W	2.68	W	2.43	W	3.60	
2.41	M	2.40	S	2.27	W	2.58	W	2.28	VW	3.50	
2.28	W	2.25	W	2.15	W	2.40	M	2.16	VW	3.44	
2.13	M	2.14	M	2.06	W	2.22	VW	2.04	VW	3.34	
1.98	W	1.98	W	1.88	W	2.12	M	1.96	VW	3.25	
1.80	W	1.85	W	1.79	W	2.06	W	1.88	VW	3.16	
1.72	M	1.72	W	1.65	W	1.95	VW	1.74	VW	2.99	
1.51	VW	1.66	W	1.55	VW	1.86	VW	1.66	VW	2.93	
1.44	VW	1.59	W	1.47	VW	1.78	VW				
1.37	VW	1.49	VW			1.70	VW				
1.25	VW	1.42	W								
1.17	VW	1.17	VW								
16. 3,4-Dimethyl- phenyl N-phenylcarbamate		17. 3,5-Dimethyl- phenyl N-phenylcar- bamate		18. 2,4,6-Tri- methylphenyl N-phenylcarbamate		19. 2- <i>tert</i> -Butyl-4- chlorophenyl N-phenylcarbamate		20. 4- <i>tert</i> -Butylphenyl N-phenylcarbamate		21. 4-Methyl-2- methylallyl)phenyl N-phenylcarbamate	
8.2	M	7.7	(3) S	8.1	S	8.5	(3) S	7.8	M	6.4	
6.7	M	5.7	(2) S	7.1	M	5.5	S	7.4	W	5.8	
5.5	S	4.76	S	6.3	M	4.83	(2) VS	6.2	M	4.97	
4.83	(3) VS	4.16	(1) VS	5.8	(2) S	4.32	(2) VS	4.56	(1) VS	4.28	(1)
4.30	(2) VS	3.70	M	4.83	(1) VS	3.65	(1) VS	3.98	(2) S	3.82	(2)
3.70	(1) VS	3.38	M	4.61	W	3.26	M	3.68	W	3.34	(3)
3.38	S	3.20	M	4.03	(3) S	3.02	M	3.25	(3) S	3.18	
3.21	M	2.88	M	3.81	S	2.89	M	3.04	M	2.96	
2.89D	M	2.55	W	3.59	S	2.68	M	2.88	W	2.67	
2.71	W	2.40	M	3.26	M	2.40	VW	2.71	W	2.51	
2.53	M	2.15	M	2.91D	M	2.26	VW	2.34	M	2.31	
2.23	M	1.89D	W	2.74	W	2.08	W	2.11	W	2.23	
2.08	VW	1.82	W	2.68	W	1.92	W	1.98	W	2.00D	
2.00	W	1.72	W	2.59	W	1.81	W	1.87	W	1.92	
1.90	M	1.63	W	2.37	M	1.64	VW	1.63	W	1.81	
1.82	W	1.43	W	2.23	W	1.52	VW	1.55	VW	1.66	
1.66	VW	1.18	VW	2.19	W					1.59	
1.58	VW	1.08	VW	2.11	VW						
1.22	VW			1.96	W						
				1.83	W						
				1.76	VW						
22. 4- <i>tert</i> -Amyl- phenyl N-phenylcar- bamate		23. 4(or 6)- <i>tert</i> -Butyl- 2-methylphenyl N-phenylcarbamate		24. 6(or 4)- <i>tert</i> -Butyl- 2-methylphenyl N-phenylcarbamate		25 <sup>b</sup> . 4(or 6)- <i>tert</i> -Bu- tyl-3-methylphenyl N-phenylcarbamate		26. 2- <i>tert</i> -Butyl-4- methylphenyl N-phenylcarbamate		27. 4-Phenylphe- N-phenylcarbamate	
8.1	W	6.6	M	7.7	VW	12.0	S	8.6	M	8.4	
6.5	(2) S	4.78D	(1) VS	6.4	(1) VS	8.1	M	7.5	W	6.9	
4.59D	(1) VS	4.28	M	6.2	VW	7.3	(3) S	6.4	M	5.8	
4.36	M	3.94	M	5.6	VW	6.3	M	5.4	(3) S	5.0	
3.79D	(3) S	3.51	(2) S	4.86	(2) VS	5.9	W	4.81	(1) VS	4.38	(1)
3.35	M	3.12	(3) S	3.94D	(3) VS	5.2	VW	4.28D	(1) S	4.18	(2)
3.09	M	2.86	M	3.40	S	4.82	(1) VS	3.63	(2) S	3.98	(3)
2.83	M	2.67	W	3.14	M	4.19	(2) S	3.27	W	3.57	
2.47	M	2.39	M	2.82	M	3.71	M	3.02	M	3.14	
2.31	W	2.28	W	2.47	M	3.54	W	2.89	M	2.93	
2.19	W	2.09	W	2.28	M	3.21	W	2.68	W	2.64	
1.99	W	1.82	W	2.07	W	2.89	W	2.23	VW	2.33D	
1.86	VW	1.62	W	1.96	W			2.11	W	2.11	
1.74	VW			1.77	VW			1.90	W	1.99	
1.65	VW			1.61	VW			1.80	W	1.84	
								1.66	VW	1.63	
								1.53	VW		
								1.43	VW		
								1.36	VW		
28. 2,6-Diacetyl-3,5- dimethylphenol		29. 2-Cyclohexyl- phenyl N-phenylcar- bamate		30. 4-Cyclohexyl- phenyl N-phenylcar- bamate		31. 2- <i>tert</i> -Amyl-4- methylphenyl N-phenylcarbamate		32. 4(or 6)- <i>tert</i> - Butyl-3-ethylphenyl N-phenylcarbamate		33. 2- <i>tert</i> -Butyl- ethylphenyl N-phenylcarbamate	
6.7D	(3) S	6.6	M	7.8	S	8.6	M	7.2	(3) S	7.3	
5.7D	M	6.1	M	6.7	S	6.5	S	5.4	W	5.7	(3)
4.78	M	4.94	(2) S	4.47D	(1) VS	5.5	(3) S	4.78	(1) VS	4.63D	(1)
4.28	M	4.26D	(1) VS	3.98	(2) VS	5.1	(1) VS	4.59	M	4.36	
3.82	(2) VS	3.55	M	3.67	M	4.30	S	3.99	(2) S	3.81	(2)
3.39	(1) VS	3.26	(3) M	3.20D	(3) S	3.65	(2) VS	3.57	M	3.62	
3.28	W	3.10	W	2.97	S	3.34	M	3.37	M	3.26	
3.12	M	2.92	W	2.70	M	3.14	M	3.04	M	3.04	
2.87	M	2.61	VW	2.53	W	2.93	M	2.68	W	2.86	
2.58D	M	2.47	M	2.41	M	2.72	M	2.47	VW	2.69	
2.41	M	2.31	VW	2.32	W	2.49	M	2.35	VW	2.55	
2.26	M	2.13	VW	2.22	M	2.27	M	2.25	VW	2.30	
2.15	VW	2.03	VW	2.11	W	2.12	M	2.08	M	2.22	
2.07	W	1.84	VW	1.66	M	1.90D	M	1.91	W	2.03	
1.98	W	1.67	VW	1.61	VW	1.84	M	1.77	VW	1.95	
1.89	W	1.62	VW	1.53	VW	1.80	M	1.55	VW	1.86	
1.84	W			1.47	VW	1.66	M			1.78	
1.71	M					1.39	VW			1.63	
						1.26	VW				
						1.22	VW				
						1.12	VW				
						1.05	VW				

<sup>a</sup> *d* = interplanar spacing in Ångströms; *I*/*I*<sub>1</sub> = estimated relative intensity; S = strong; M = medium; W = weak; V = very. Three strongest lines indicated in decreasing order of intensity by (1), (2), and (3). For explanation of D see text.

<sup>b</sup> Data obtained using a camera of 171.9-mm. effective diameter.



Table II. Powder Diffraction Data<sup>a</sup> (Cont'd)[illegible]

In instances the pertinent data in Tables I and II refer to the compound itself and the footnote to Table I points out these exceptions. In a very few cases, the specific structure of the phenol was not known, and for these compounds an alternative structure is given in parentheses and the boiling point of the compound is indicated. For example, two mono-*tert*-butyl derivatives of *o*-cresol are probable in which the *tert*-butyl radical is in either the 6 or 2 position. To the isomer boiling at 135° C. at 20 mm., the formula 2-methyl-4(or 6)-*tert*-butylphenol was assigned, while to the one boiling at 123° C. at 20 mm. was given the formula 2-methyl-6(or 4)-*tert*-butylphenol. Phenols which would not form aryl *N*-phenylcarbamates by reaction with phenyl isocyanate were, in general, of the type in

which both positions ortho to the hydroxyl were occupied by large groups which hindered the activity of the hydroxyl hydrogen. Specifically, these were 2,6-diacetyl-3,5-dimethylphenol, 2,6-di-*tert*-butyl-4-chlorophenol, 2,6-di-*tert*-butyl-4-cyclohexylphenol, 2,4,6-tri-*tert*-butylphenol, 2,6-di-*tert*-butyl-4-methylphenol, and 2,6-di-*tert*-butyl-4-ethylphenol. 2-Nitrophenol reacts only to a very limited extent with phenyl isocyanate (6) and for this reason the properties of its derivative are not included in this paper.

The nonreactivity of 2,6-di-*tert*-butyl substituted phenols is interesting in that it makes possible the removal of admixed 2-*tert*-butyl substituted phenols upon treatment of such mixtures with phenyl isocyanate. Advantage of this fact was taken



in the preparation of 2-*tert*-butyl-4-cyclohexylphenyl-*N*-phenylcarbamate from a mixture of 2-*tert*-butyl-4-cyclohexylphenol and 2,6-di-*tert*-butyl-4-cyclohexylphenol. The aryl *N*-phenylcarbamate obtained had a sharp melting point indicating its purity, and its elementary analysis (calculated: C, 78.59; H, 8.32; found: C, 78.54; H, 8.19) indicated that only the mono-*tert*-butyl derivative reacted with the phenyl isocyanate.

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#### LITERATURE CITED

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## Bomb Furnace for Carius Digestion

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THE oxidation of organic material in a sealed glass tube at high temperatures and pressures, as first used by Carius for the determination of halides (1), is still used for the determination of halides, sulfur, and nitrogen. The pressure tubes are made of heavy Jena glass or Pyrex; they are carefully flame-sealed, avoiding any strain in the glass, and are heated to temperatures as high as 300° C. in a bomb furnace. The pressure developed within the tubes is so great that they frequently explode, causing considerable damage (2). In carrying out halide determinations in this laboratory, there have been several such explosions. The author has therefore devised a bomb furnace in which the danger of explosion is eliminated.

In principle, the glass pressure tube is heated in an atmosphere whose pressures approximate the pressure developed within the tube. A diagram of the furnace (built by the Bigelow Boiler Works, New Haven, Conn.) is shown in Figure 1. It consists of a steel tube 70 cm. in length, 4 cm. in diameter, and closed at one end. The wall of the steel tube is 3 mm. thick. The open end has a flange measuring 13 cm. in diameter and 3 cm. in thickness, to which a cap of similar dimensions can be bolted. The cap has attached to it a steam pressure gage reading up to 910 kg. (2000 pounds), a safety valve, and a manual valve. The furnace is built to operate safely up to 910 kg. (2000 pounds) and the safety valve is adjusted to open at 700 kg. (1500 pounds). The steel tube is supported in a vertical position by a metal frame and is heated at its lower end by vertical gas burners.

To use the furnace, water is placed in the vertical steel tube to a depth of about 20 cm. The sealed glass bomb, about 25 cm. in length, is suspended by a short piece of cord tied at one end to a hook drawn out at the sealed end of the tube, and at the other end to a short metal bar laid across the mouth of the steel tube. The glass bomb must not dip into the water and must not come into contact with the wall of the steel tube. This is easily accomplished if the furnace is perfectly vertical. A gasket is placed on the flanged open end of the furnace and the cap is bolted into position. With the manual valve open, the gas burners are lighted and when the vapor starts to escape from this valve, it is closed. Heating is continued until the desired temperature is reached as estimated by the pressure on the gage. The flame is then adjusted to maintain this pressure. At the end, the flame is turned off and the furnace is allowed to cool until the pressure reading is not more than a few pounds. The manual valve is then opened and the cap is removed.

The author has carried out many Carius digestions with this furnace, using Pyrex tubes of ordinary thickness, without losing one. Although the unit illustrated holds only 1 pressure tube, a multiple unit can be easily constructed. Besides the determina-

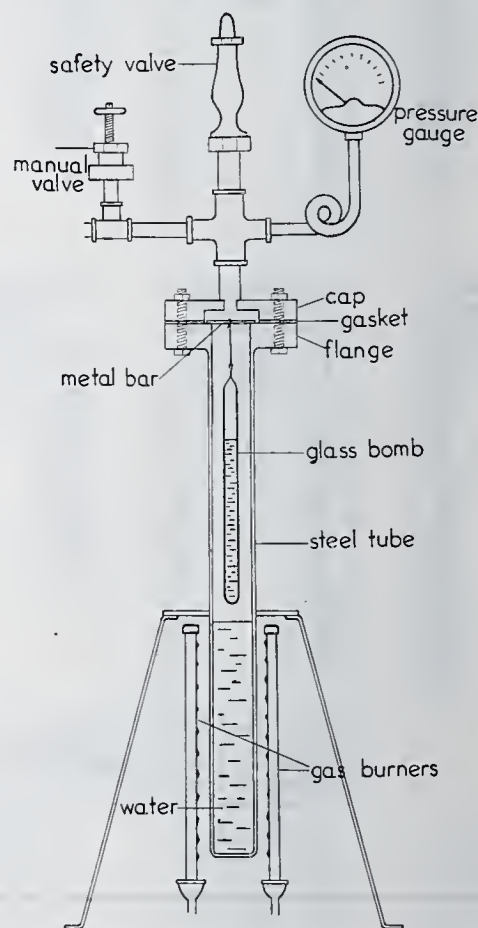


Figure 1. Diagram of Furnace

tions of halides, sulfur, and nitrogen, many other procedures used in laboratories, such as aminization, must be carried out in glass bombs at high temperatures. For such purposes, the type of furnace described here is useful in eliminating the danger of explosion.

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# Direct Photometric Determination of Silicon in Copper-Base Alloys

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A new method for the direct photometric determination of silicon in copper-base alloys offers considerable advantage over the regular gravimetric method in speed and simplicity of operation. Determinations of silicon in a group of manganese bronze samples containing up to 0.15% of silicon show good agreement with results obtained by the regular gravimetric method. The optimum conditions for the development and evaluation of the silicomolybdate color complex in the presence of copper are discussed. A method for overcoming the interference of phosphorus is described.

THE usual gravimetric determination of silicon in copper-base alloys (2) is a rather lengthy and exacting procedure. Two dehydrations must be made to ensure complete separation of the silicon and where, as is often the case, insoluble silicides remain undecomposed after the first dehydration, these must be fused with sodium carbonate and carried through two more dehydrations. These multiple dehydrations of the large amount of salts resulting from a 5-gram sample must be made slowly and carefully to avoid spattering. Besides the time consumed by dehydration, considerably more time must be spent in filtering, igniting, weighing, and volatilizing the separated silica and reweighing the platinum crucible.

In the present emergency, when large volumes of samples must be handled daily and results reported quickly, a simpler and more rapid method for this determination is highly desirable. A photometric method seemed to offer the best possibility along these lines.

A survey of the literature revealed that practically all the proposed colorimetric methods for the determination of silicon (3, 20) depend upon the formation of a silicomolybdate complex from the reaction of silicic acid and ammonium molybdate in a moderately acid solution. This method is often credited to Went and Wandenbuleke (3) in the recent literature, but the reaction was used for the colorimetric determination of silica as long ago as 1898 by Jolles and Neurath (6). The method in various modifications has been used for the determination of silicon in fresh water (3, 6, 9), in sea water (13, 18, 19), in boiler-d water (15), in tissue (8), in iron and steel (11), and in aluminum- and magnesium-base alloys (1, 4, 5, 12). No reference could be found to the use of this reaction for the determination of silicon in copper-base alloys.

That the reaction has not been so employed is probably due to difficulty in obtaining complete solution of the silicon when binary acids are used for dissolving these alloys. The thought occurred that if the samples were dissolved by adding a little hydrofluoric acid to the regular dissolving acid, and the excess hydrofluoric acid was inactivated by the addition of boric acid (7), it should be possible to obtain a solution of the sample which could be treated directly with ammonium molybdate to develop the silicomolybdate color complex. Experiment showed this to be true. Solution of the sample must, of course, take place in a platinum container and all contact with glass must be avoided until after the addition of the boric acid, as hydrofluoric acid attacks glassware.

Presumably the following reactions take place: Silicides are dissolved by action of the hydrofluoric acid, forming silicon tetrafluoride which reacts with water to form silicic acid and hydrofluoric acid. Since the amount of silicic acid is small, it does not precipitate. The boric acid reacts with the excess hydrofluoric acid, forming fluoboric acid (7). Experiment has shown that

the silicon in fluosilicic acid reacts with ammonium molybdate to produce the same color as an equivalent amount of silicon in silicic acid. The hydrofluoric acid also appears to form stable complexes with tin and iron when these are present in the sample, preventing the precipitation of metastannic acid and the formation of the colored iron molybdate complex noted by Thayer (18). Free hydrofluoric acid prevents, or greatly retards, the formation of the silicomolybdate complex.

## PROPOSED METHOD

**SOLUTIONS AND REAGENTS REQUIRED.** Dilute Nitric Acid (1 to 2). Dilute 1 volume of reagent nitric acid with 2 volumes of water.

**Hydrofluoric Acid (48%),** reagent grade. Even the best grades of hydrofluoric acid appear to contain a small amount of fluosilicic acid.

**Boric Acid (saturated solution).** Dissolve 65 grams of reagent boric acid crystals,  $H_3BO_3$ , in 1 liter of hot water. Cool to room temperature.

**Ammonium Molybdate (10%).** Dissolve 100 grams of reagent ammonium molybdate crystals,  $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ , in hot water. Cool and dilute to 1 liter. Filter if the solution is not clear.

**Citric Acid (10%).** Dissolve 100 grams of reagent citric acid crystals,  $C_6H_8O_7 \cdot H_2O$ , in water and dilute to 1 liter.

**Standard Silicate Solution** (1 ml. = 0.0001 gram of silicon). Fuse 0.2141 gram of pure anhydrous silica,  $SiO_2$ , with 2 grams of sodium carbonate in a platinum crucible. Heat at slightly above fusion temperature for about 15 minutes, cool, and dissolve the melt in warm water, using a platinum dish for a container. Cool the solution and transfer to a 1000-ml. volumetric flask. Dilute to the mark and mix thoroughly. Store the solution in a wax or hard-rubber bottle.

**PREPARATION OF CALIBRATION CURVE** (for alloys containing up to 0.20% silicon). Weigh portions of high-purity copper equivalent to the amount of copper ( $\pm 25$  mg.) present in a 1-gram sample of the alloy under test. Very fine pieces of metal (35-mesh) and light, feathery drillings should be avoided, as they react too vigorously with the dissolving acid. Transfer to platinum crucibles of at least 20-ml. capacity fitted with covers. Somewhat larger crucibles are preferable if available. To each portion of metal add 10 drops of hydrofluoric acid (0.3 to 0.4 ml.) followed by an amount of dilute nitric acid (1 to 2) equivalent to 0.6 ml. for each 100 mg. of metal plus 6 ml. in excess. Cover the crucibles and let stand until the vigorous reaction has subsided, when they may be placed on the steam plate to complete solution. With the aid of a long-stemmed hard-rubber or plastic funnel, transfer the contents of the crucibles to 200-ml. volumetric flasks containing 25 ml. of boric acid solution. Rinse the crucibles and sides of the flasks and immediately swirl the flasks to mix the solutions thoroughly. From a microburet add amounts of standard silicate solution to cover the desired range of silicon in steps of 0.2 mg. Cool the solutions to room temperature and add 10 ml. of ammonium molybdate solution to each. Dilute to the mark and mix thoroughly. Let the solutions stand for 15 minutes and read the transmission or relative density of the color with a photometer at approximately 410 millimicrons. Plot the photometer readings against milligrams of silicon, or per cent of silicon. The curve approximates a straight line. Alternately, a calibration curve may be plotted by using several carefully analyzed samples of the alloy under test as standards, covering as wide a range of silicon content as possible.

While this method of calibration automatically compensates for the reagent blank, this blank may vary for different lots of reagents, and it is desirable to run either a synthetic standard or an analyzed sample of the alloy under test to determine whether or not a correction should be applied each time a new lot of reagents is used.

**PROCEDURE FOR SAMPLES.** Treat a 1- to 1.0050-gram sample of the alloy under test exactly as described above, omitting addition of the standard silicate solution. Read per cent silicon directly from calibration curve.



Table I. Silicon in Ounce Metal

(National Bureau of Standards Standard Sample 124, per cent composition: copper, 83.77; zinc, 5.46; tin, 4.69; lead, 4.78; iron, 0.38; nickel, 0.45; antimony, 0.23; sulfur, 0.071; phosphorus, 0.037; silicon, 0.075. Evelyn photoelectric colorimeter, filter 400)

Silicon, Per Cent				
Citric Acid Added Ml.	Colorimeter Reading	Colorimetric	B. of S. certificate	Difference
0	30.75	0.086	0.075	+0.011
0	31.00	0.085	0.075	+0.010
5	35.50	0.073	0.075	-0.002
5	35.00	0.074	0.075	-0.001
5	35.00	0.074	0.075	-0.001
5	35.00	0.074	0.075	-0.001
5	34.75	0.075	0.075	0.000
5	34.50	0.076	0.075	+0.001

DISCUSSION

The method of solution causes no significant loss of silicon volatilized as silicon tetrafluoride, provided the crucibles are covered and the pieces of sample metal are not so fine as to cause an exceedingly vigorous reaction which would bring the metal to the surface of the solution. Samples of silicon bronze containing as high as 15 mg. of silicon have been dissolved in this way with no significant loss of silicon.

The amount of hydrofluoric acid added in dissolving the sample should be kept to the minimum necessary for complete solution of the silicon (and tin if present); 0.3 to 0.4 ml. is ample for amounts of silicon up to 15 mg. This amount of hydrofluoric acid (48% reagent grade) may contain as much as 0.1 mg. of silicon as fluosilicic acid.

The amount of boric acid solution used is not critical, provided enough is present to react with the excess hydrofluoric acid. Using the technique described in the proposed method, 25 ml. of a saturated solution of boric acid are ample for inactivating the excess hydrofluoric acid. If desired, 1 gram of dry boric acid crystals may be added directly to the sample contained in the platinum crucible after solution is complete, the crucible being heated gently until the boric acid dissolves. Alternately, the solution of the sample may be mixed with a saturated boric acid solution in a platinum dish. The last two techniques obviate the necessity for using a funnel of nonsilicate material in transferring to the volumetric flask.

The amount of diluted nitric acid (1 to 2) used in dissolving the sample affects the color developed considerably; 12 to 14 ml. of acid for a 1-gram sample diluted to 200 ml. give the most intense color. The optimum pH for maximum color intensity was found to be 0.75 to 1.25, using the quinhydrone electrode. This pH is somewhat lower than has been recommended by Knudson *et al.* (9) but is in substantial agreement with the findings of Schwartz (15).

At the pH recommended, the full color of the silicomolybdate complex develops almost immediately and does not fade appreciably for at least 30 minutes.

Eight to 10 ml. of 10% ammonium molybdate solution in a total volume of 200 ml. give the maximum color development. Less molybdate retards the color development, but amounts in excess of 10 ml. do not appreciably increase the intensity of the color.

Investigation of the spectral transmittance of each of the two colored components of the copper nitrate-silicomolybdate solution showed that the maximum transmittance of the copper nitrate occurred below 430 millimicrons and that the maximum absorption of the silicomolybdate complex occurred at 410 to 420 millimicrons. These findings indicate the use of a filter which has a maximum transmission between 400 and 430 millimicrons. Schwartz and Morris (16) have recommended a filter transmitting in the neighborhood of 410 millimicrons for maximum sensitivity in reading the color values of silicomolybdate solutions.

The maximum sensitivity of the silicomolybdate color read at

approximately 410 millimicrons is obtained when the concentration of silicon is not greater than 1.0 mg. per 100 ml. While detailed procedure is given only for those copper-base alloys which contain not more than 0.20% silicon, and the recommended amounts of reagents are based upon a 1-gram sample diluted 200 ml., preliminary experiments with silicon bronze alloys indicate that by suitable dilution or the use of a smaller sample bring the concentration of the silicon into the most sensitive range, copper-base alloys containing up to 3.50% of silicon may be analyzed by this method.

A few elements other than silicon also form colored complexes with molybdic acid, notably phosphorus, germanium, and arsenic (10). Of these, the arsenic complex is not formed at room temperature, and germanium is a very rare constituent of copper-base alloys. Infrequently a small amount of phosphorus may occur as an impurity in copper-base alloys containing silicon. Under the analytical conditions described in the proposed method, a given amount of phosphorus develops somewhat less than half the color intensity of an equal amount of silicon. For practical purposes, amounts of phosphorus less than 0.01% appear to have no significant influence on the silicon determination. Where phosphorus is present in amounts large enough to cause interference, the colored phosphorus complex may be selectively destroyed by the addition of citric acid (15). Oxalic acid which has been recommended for this purpose by Schwartz (15) cannot be used in the presence of copper, owing to the solubility of copper oxalate.

Table II. Silicon in Manganese Bronze

(Approximate per cent composition: copper, 60; tin, up to 2.50; iron, 0.03; manganese, 0.03; silicon, up to 0.15; zinc, balance. Evelyn photoelectric colorimeter, filter 400)

Sample No.	Colorimeter Reading	Silicon, Per Cent		Difference
		Colorimetric	Gravimetric	
8,194	46.50	0.050	0.050	0.000
	46.00	0.051		+0.001
13,520	36.25	0.072	0.067	+0.005
	36.75	0.070		+0.003
13,519	35.50	0.073	0.070	+0.003
	34.75	0.075		+0.002
7,725	35.25	0.074	0.071	+0.003
	35.25	0.074		+0.000
8,159	36.25	0.071	0.073	-0.002
	35.75	0.072		-0.001
4,627	34.25	0.076	0.078	-0.002
	35.25	0.074		-0.002
13,518	28.00	0.095	0.092	+0.003
	27.25	0.098		+0.003
7,721	28.75	0.093	0.093	0.000
	28.25	0.094		+0.001
8,907	26.00	0.102	0.101	+0.001
	25.00	0.106		+0.004
7,969	23.25	0.114	0.112	+0.002
	22.50	0.117		+0.003
6,952	23.25	0.114	0.113	+0.001
	23.00	0.115		+0.001
7,967	24.00	0.110	0.116	-0.006
	22.75	0.116		0.000
8,201	20.00	0.130	0.131	-0.001
	21.00	0.125		-0.005
8,167	18.50	0.140	0.133	+0.007
	18.25	0.141		+0.001
8,165	16.25	0.155	0.154	+0.001
	17.00	0.150		-0.005

To determine silicon in the presence of phosphorus, the sample is treated as described under Proposed Method until the ammonium molybdate has been added, then the sample solution is diluted to 175 ml. and let stand for 10 minutes. Five milliliters of 10% citric acid are added, diluted to the mark, and mixed thoroughly, and the color value is read at once.

Under the above conditions the phosphorus complex is bleached almost at once, while the silicon complex is not significantly affected for several minutes; 5 ml. of 10% citric acid are sufficient to destroy the color developed by 2.5 mg. of phosphorus.

Samples of National Bureau of Standards ounce metal (Standard Sample 124) containing 0.037% phosphorus and 0.075% silicon were analyzed colorimetrically for silicon, both with and without additions of citric acid. Results are shown in Table I.

RESULTS ON COMMERCIAL COPPER-BASE ALLOYS

Samples of manganese bronze, having a composition of approximately 60% copper, up to 2.50% tin, 1% iron, 0.03%



anganese, up to 0.15% silicon, and the balance zinc, were analyzed for silicon both by the regular gravimetric method using gram samples and by the colorimetric method here presented. Results are shown in Table II. Good agreement between the silicon values obtained by the two methods is indicated. Individual determinations were usually reproducible within 5%. All photometric readings were taken with an Evelyn photoelectric colorimeter, using the Evelyn No. 400 filter which has a transmission range of 380 to 430 millimicrons.

#### ACKNOWLEDGMENT

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## Determination of Germanium in Steel

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A gravimetric method for the analysis of germanium in steel which is free from any known interference, consists of separation of germanium by distillation of the tetrachloride, precipitation of germanium in the distillate with tannin, and ignition to the oxide. This method yielded entirely satisfactory results, the average error being of the order of 0.001% in steels containing up to 0.5% of germanium. Using synthetic standards, it was shown to be applicable to steels containing up to 0.5% of germanium, or up to 10% of germanium if a 1-gram sample is used instead of 10 grams. With a single distillation apparatus, an average of four determinations a day can be completed.

DURING an investigation at the Naval Research Laboratory, it was necessary to analyze several samples of steel for germanium. No method for the determination of germanium in steel was found in the literature; however, by suitable modifications of the methods given for the analysis of residues and ashes, it was possible to work out a satisfactory method for determining moderate amounts of germanium in steel.

The most convenient method for the separation of germanium from practically all other elements normally present in steel is distillation of germanium tetrachloride from a hydrochloric acid solution; this method was devised by Buchanan (5) for separating germanium from germaniferous zinc oxide residues. Tin and arsenic may be expected to accompany germanium into the distillate, but contamination by tin can be avoided by adding sulfuric acid to the solution before distillation (9). Contamination by arsenic can be completely eliminated through the method of Dennis and Johnson (7) by distilling in a current of chlorine gas using an efficient still head. Under these conditions arsenic chloride is oxidized to the less volatile pentachloride and a separation from germanium may be obtained.

However, the separation of germanium from moderate amounts of arsenic is unnecessary, since Davies and Morgan (6) have shown that germanium may be satisfactorily determined in the presence of arsenic by precipitation of the former with tannic acid. The oldest gravimetric method for germanium is Winkler's, in which germanium sulfide is precipitated from strong acid

solution, treated with nitric acid, and ignited to the oxide (13). Because of the danger of loss in this procedure, Johnson and Dennis (11) prefer to dissolve the sulfide in ammonium hydroxide and then to oxidize with hydrogen peroxide; in either case, complete precipitation of germanium as the sulfide requires 24 hours or longer, and arsenic is also precipitated. Other methods are precipitation of the 8-hydroxyquinolate of germanomolybdic acid (3, 4) and colorimetric determination of the blue reduction product obtained by treating germanomolybdic acid with ferrous sulfate (10). The colorimetric method is subject to interferences from traces of arsenic, silica, and phosphorus.

Preliminary work indicated the superiority of the tannin method over other published procedures for the determination of germanium. Slow evaporation to dryness of a pure germanium solution containing hydrofluoric, hydrochloric, perchloric, and sulfuric acids, followed by ignition to the oxide (2) gave accurate results in this laboratory, but was too time-consuming. Determination of germanium by precipitation and weighing as magnesium orthogermanate (12) gave high results in preliminary experiments, as has been noted by others (6).

It seemed best to separate germanium, with a small amount of arsenic, from the other elements present in steel by distillation from a solution containing hydrochloric and sulfuric acids, and then to precipitate the germanium in the distillate with tannic acid.

#### EXPERIMENTAL

Mixtures of 10 grams of various germanium-free steels with weighed amounts of Adam Hilger's "spectroscopically standardized" grade of germanium metal, ground to pass a 100-mesh sieve, were analyzed by the procedure described below. The results obtained are shown in Table I.

The germanium-bearing steels under investigation were analyzed by the same method (Table II).

#### RECOMMENDED PROCEDURE

Transfer 10.00 grams of the germanium steel sample to a 500-ml. round-bottomed flask with standard taper neck and add a mixture of 10 ml. of nitric acid and 100 ml. of 1 to 4 sulfuric acid. When most of the action has ceased, boil the mixture



Table I. Analysis of Steel-Germanium Mixtures

(100-mesh metallic germanium + 10 grams of steel)		
Germanium Taken	Germanium Found	Error
Gram	Gram	Gram
0.0024	0.0022	-0.0002
0.0051	0.0051	0.0000
0.0051	0.0053	+0.0002
0.0127	0.0128	+0.0001
0.0178	0.0177	-0.0001
0.0259	0.0259	0.0000
0.0401	0.0402	+0.0001
0.0466	0.0464	-0.0002
0.0510	0.0511	+0.0001
		Av. 0.0001

Table II. Analysis of Germanium Steels

Sample	Germanium Added to Steel during Melting, Approximate %	Germanium Found %
GHB	0.053	0.051
		0.052
		0.053
GHC	0.11	0.102
		0.102
GHD	0.17	0.161
		0.162
		0.160
		0.161
		0.161

gently for 30 minutes to ensure complete solution of the germanium. Cool somewhat and add 5 grams of copper turnings to destroy the excess of nitric acid. Boil 3 minutes to expel oxides of nitrogen.

Wash down the inside of the flask with enough water to make the total volume about 150 ml., and cool the solution in an ice bath. Add 200 ml. of hydrochloric acid and immediately connect to the distilling apparatus (Figure 1). An efficient fractionating device, the Widmer column, is included in the distilling apparatus in order to minimize contamination by higher-boiling metal chlorides. Details of the construction of the column are given by Adkins and McElvain (1). Two precautions are taken against suck-back during distillation: a pressure-equalization pinchcock, *C*, is provided at the top of the fractionating column and a safety bulb, *B*, 5 cm. (2 inches) in diameter is included in the adapter.

Start the distillation, adjusting the rate of heating so that the constant-boiling hydrochloric acid distills over at the rate of one drop in about 5 seconds, and collect 20 to 30 ml. of distillate in 100 ml. of ice-cold water in the receiver. Nearly all the germanium tetrachloride (b.p. 86° C.) comes over before any of the constant-boiling acid.

Disconnect the apparatus, wash the adapter with a jet of water, and add 2 grams of hydroxylamine hydrochloride to the distillate to reduce any oxidizing substances. With stirring, add 30 ml. of a fresh 5% tannin solution, and then a few drops of methyl red indicator. Add ammonia until the solution is alkaline, then make it barely acid by dropwise additions of sulfuric acid and add 10 drops in excess. Davies and Morgan (6) state that acidity as high as 1.0 *N* sulfuric acid is permissible. The 10-drop excess in a volume of 200 ml. gives an acidity of about 0.08 *N*, which seems to be well within the safe limits. Heat the mixture to incipient boiling and allow to stand until the flocculent precipitate has settled and the solution is cool.

Filter the precipitate through a 15-cm. No. 40 Whatman paper, washing until completely free of chlorides with a wash water containing 50 grams of ammonium nitrate, 5 grams of tannin, and 5 ml. of nitric acid per liter. Ignite cautiously at first in a weighed platinum crucible, then at 600° C. for about an hour. Allow to cool, treat with 5 drops of sulfuric acid and 3 ml. of nitric acid, and evaporate to dryness to destroy most of the remaining carbon. Ignite again below 600° C. until all carbon is burned off and finally ignite the white residue at 900° to 1000° C. for 10 minutes and weigh as germanium dioxide.

Correct for the weight of a blank obtained by carrying 10 grams of germanium-free steel through the procedure, and multiply the weight of germanium dioxide in grams by 6.941 to obtain the percentage of germanium in the steel.

#### DISCUSSION

Preliminary work showed that hydrochloric acid could be substituted for sulfuric acid in the tannin precipitation pro-

cedure, and that considerable variations in the amounts of hydrochloric and nitric acids present in the distillate caused no difficulty.

Entirely satisfactory results were obtained through the use of the recommended procedure, which gave a reproducibility of 0.002% in comparison with another recent method for germanium (4), the reproducibility of which is stated as 0.02%; but extreme care must be taken to wash the precipitate completely free of chlorides, or loss of germanium will occur upon ignition.

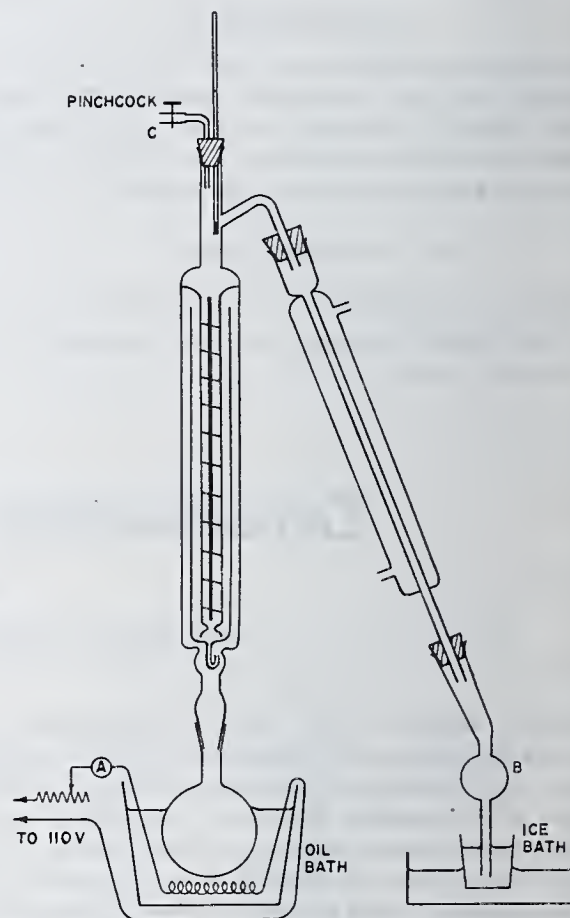


Figure 1. Distilling Apparatus

The ignition must be performed at 900° to 1000° C. Above this range, germanium dioxide begins to volatilize; below it, results are too high, perhaps because of incomplete decomposition of germanium sulfate.

Germanium tetrachloride vapors are reported to be difficult to condense (2, 8). Several trials were made for the purpose of determining whether germanium was lost because of incomplete condensation, but no germanium was ever found in the wash water of a Milligan gas-washing bottle through which the gases were led after bubbling through the ice water.

An effort was made to determine whether any germanium was present in the insoluble residue remaining after solution and distillation of a germanium steel.

This insoluble residue was filtered off, washed thoroughly first with hot 1 to 9 hydrochloric acid and finally with hot water, ignited, and fused with sodium carbonate and sodium nitrate. The melt was dissolved in water and then acidified with sulfuric acid, 200 ml. of hydrochloric acid were added, and the solution was distilled and analyzed for germanium as usual. With a 10-gram sample of steel GHD, no germanium was found in the acid-insoluble residue. When 50-gram samples were treated in the same manner, GHB was found to contain 0.002% and GHC 0.003% of acid-insoluble germanium.

#### ACKNOWLEDGMENT

The author gratefully acknowledges the invaluable suggestions of Louis Singer, under whose guidance this work was performed.



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# Determination of Magnesia in Magnesite and Dolomite

## A Potentiometric Method

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The magnesia content of magnesites and dolomites may be determined by potentiometric titration, using a glass electrode. Excess acid present on solution of the sample is neutralized with analytical reagent calcium carbonate. The titration is made in a hot 50% solution of alcohol, with standard carbonate-free caustic. Weak bases such as iron, aluminum, and titanium normally present in magnesite and dolomite do not interfere. The method attains an accuracy which compares favorably with that of the ammonium phosphate procedure for magnesium and is much less time-consuming.

The determination of magnesia in magnesite and dolomite by precipitation as magnesium ammonium phosphate is tedious and time-consuming. Hildebrand and Harned (1) attempted to determine magnesia in limestone potentiometrically by means of a hydrogen electrode. Zhukov and Gortikov (3) and Treadwell and Bernasconi (2) investigated a potentiometric system for estimating magnesia, employing antimony as the indicator electrode.

The potentiometric method described in this paper makes use of a glass electrode-saturated calomel system. The magnesium ion is titrated in a hot 50% solution of alcohol with standard sodium hydroxide. The excess acid normally present after solution of the sample is neutralized with carbonate-free caustic or with analytical reagent calcium carbonate. The latter markedly minimizes the error due to weak bases. All titrations are made using a Leitz Titrimeter Model G & D. No absolute potential values are listed in this investigation.

### REAGENTS

Standard sodium hydroxide (carbonate-free), 1.054 *N*. Standard magnesium chloride solution (10.554 grams of pure magnesium per liter). Calcium carbonate, analytical reagent. Thymolphthalein indicator, 0.1% alcoholic solution. Ethyl alcohol, 95%.

### PROCEDURE

Weigh a 2.000-gram sample into a 400-ml. beaker, add 20 ml. 1 to 1 perchloric acid, cover the beaker with a Speedyvap watch glass, and take to dryness on a hot plate. Add 100 ml. of distilled water and 10 drops of concentrated hydrochloric acid, boil briefly to aid in dissolving the salts, and add analytical reagent calcium carbonate to neutralize the free hydrochloric acid, usually boiling to remove the excess carbon dioxide. (If sodium hydroxide is used, neutralize electrometrically at room temperature.) Add 100 ml. of ethyl alcohol and 2 ml. of thymolphthalein indicator, and transfer to the electrometric titrator, keeping the solution near boiling with the aid of an electric hot plate. Turn the mechanical stirrer and add a few milliliters of standard

hydroxide. Permit the solution to come to equilibrium and adjust the titrator, using the maximum sensitivity. Continue the titration.

The addition of the remainder of the sodium hydroxide affects the galvanometer reading only slightly until the end point is reached. The thymolphthalein indicator warns of the nearness of the end point by turning a deep blue. Add the titrant in 0.1-ml. portions near the end of the titration for most satisfactory results. The greatest deflection of the galvanometer for an addition of 0.1 ml. of titrant may be taken as the end point. If more accuracy is desired, a graph of the end point may be made. The latter method was employed in this paper.

### DISCUSSION

Table I shows the standardization of a carbonate-free caustic solution using a standard magnesium chloride solution, which was prepared from pure magnesium metal dissolved in dilute hydrochloric acid. By this method, the caustic was found to be 1.054 *N*. Standardized against C.P. potassium acid phthalate, its normality was 1.053. The results in this paper are based on the former value.

The excess hydrochloric acid is neutralized potentiometrically, prior to the titration of the magnesium ion, by the use of a carbonate-free sodium hydroxide solution or by the addition of a slight excess of analytical reagent calcium carbonate. The latter reagent proved superior and is considered standard for this procedure.

Table II shows that stick antimony may be used as the indicator electrode without interference from the calcium ion in the titration of magnesium chloride solutions. While results re-

Table I. Potentiometric Standardization of a Caustic Solution (Carbonate-Free)

(Showing effect of calcium on glass indicator electrodes. Magnesium present, 0.5277 gram)

Caustic Used, Ml.	Indicator Electrode	Neutralizing Reagent
41.17	Antimony	Sodium hydroxide
41.22	Antimony	Sodium hydroxide
41.08	Antimony	Sodium hydroxide
41.18	Antimony	Sodium hydroxide
41.22	Glass	Sodium hydroxide
41.26	Glass	Sodium hydroxide
41.18	Glass	Sodium hydroxide
41.13	Glass	Calcium carbonate
41.20	Glass	Calcium carbonate
41.20	Glass	Calcium carbonate
41.15	Glass	Calcium carbonate
41.20	Glass	Calcium carbonate
41.20	Glass	Calcium carbonate
41.20	Glass	Calcium carbonate



Table II. Electrometric Titration of Magnesium Chloride<sup>a</sup>

(In the presence of calcium chloride, using antimony as the indicator electrode, magnesium present, 0.5277 gram)

Calcium Present Gram	Magnesium Recovered Gram	Magnesium Error Gram
0.200	0.5289	+0.0012
0.200	0.5277	0.0000
0.200	0.5279	+0.0002
0.400	0.5277	0.0000
0.400	0.5279	+0.0002
0.400	0.5277	0.0000
0.600	0.5279	+0.0002
0.600	0.5277	0.0000
0.600	0.5277	0.0000

<sup>a</sup> Excess hydrochloric acid present neutralized electrometrically with carbonate-free sodium hydroxide.

recorded in this table are excellent, the antimony indicator electrode frequently shows a tendency to drift at the end point. It gives unreliable results (not cited) in the presence of certain other ions studied in this investigation.

The analytical reagent calcium carbonate effectively precipitates the weak bases of titanium, aluminum, and ferric iron, making possible the direct titration of magnesium. In most instances, it is not necessary to filter the solution after neutralization with calcium carbonate. If this becomes necessary, however, the weak bases precipitated in this manner usually filter well, even permitting the use of suction. Appreciable amounts of aluminum, sulfate, or phosphate ions make filtration necessary. The phosphates normally occurring in magnesite and dolomite have no deleterious effect on the final results. Perchlorate, nitrate, and chromate ions are without influence if the determination is made using a glass indicator electrode. If manganese is present in quantities greater than 5 mg., it must be oxidized to manganese dioxide by the addition of bromine water. After neutralization with calcium carbonate, the solution is boiled until the excess bromine is removed.

Table III. Effect of Metallic Ions on Potentiometric Estimation of Magnesium (as Chloride) Using a Glass Electrode

(Magnesium present, 0.5277 gram)

Iron Present Gram	Aluminum Present Gram	Manganese Present Gram	Titanium Present Gram	Chromium Present Gram	Magnesium Found Gram
0.001	....	....	....	...	0.5277
0.001 <sup>a</sup>	....	....	....	...	0.5277
0.005	....	....	....	...	0.5281
0.005 <sup>a</sup>	....	....	....	...	0.5277
0.010	....	....	....	...	0.5270
0.010 <sup>a</sup>	....	....	....	...	0.5274
0.025	....	....	....	...	0.5268
0.050 <sup>a</sup>	....	....	....	...	0.5284
0.050	....	....	....	...	0.5289
...	0.005	....	....	...	0.5270
...	0.010	....	....	...	0.5277
...	0.015	....	....	...	0.5270
...	0.020	....	....	...	0.5277
...	0.020	....	....	...	0.5264
...	0.025	....	....	...	0.5264
...	0.025	....	....	...	0.5251
...	....	0.0011	....	...	0.5264
...	....	0.0022	....	...	0.5272
...	....	0.0055	....	...	0.5283
...	....	0.0110	....	...	0.5328
...	....	0.0110 <sup>b</sup>	....	...	0.5264
...	....	0.0100 <sup>b</sup>	....	...	0.5277
...	....	0.0100 <sup>b</sup>	....	...	0.5277
...	....	0.0100 <sup>b</sup>	....	...	0.5289
...	....	....	0.005	...	0.5277
...	....	....	0.010	...	0.5277
...	....	....	0.025	...	0.5277
...	....	....	....	0.002	0.5289
...	....	....	....	0.005 <sup>a</sup>	0.5277
...	....	....	....	0.010	0.5280
0.005	0.0003	....	0.0001	...	0.5277
0.005	0.0005	....	0.0010	...	0.5267
0.010	0.0025	....	0.0010	...	0.5277
0.010	0.0050	....	0.0050	...	0.5277
0.025	0.0125	....	0.0100	...	0.5277
0.025	0.0050	0.002	0.0050	0.002	0.5277
0.025	0.0050	0.002	0.0050	0.002	0.5277

<sup>a</sup> Excess hydrochloric acid neutralized with standard caustic (carbonate-free) using electrometric titrator. Analytical reagent calcium carbonate used to neutralize all other samples.<sup>b</sup> Manganese oxidized with bromine water. Calcium carbonate then added and excess bromine removed by boiling.

Table III shows the effect of individual elements on the determination of magnesium. The results are based on a different electrometric end point, which appears to give greater precision and accuracy than titrating to a given value on the galvanometer scale, as is often recommended.

Table IV shows a comparison of potentiometric and gravimetric (magnesium ammonium phosphate) results for magnesite in a series of magnesite samples. The fourth column records the total percentage for all elements found in a sample of magnesite with which the gravimetric value for magnesia was used and the fifth column represents the total percentage using the potentiometric value.

It is believed that the accuracy of this method compares favorably with that of the ammonium phosphate procedure for magnesia. The time required to make a single determination or series of analyses is greatly reduced. In general, it is not essential to make a separation from elements commonly associated with magnesite and dolomite.

Table IV. Potentiometric and Gravimetric (Magnesium Ammonium Phosphate) Results on Magnesite Samples

Sample No.	Magnesia Found		Total for Magnesite Analysis	
	Gravimetric %	Electrometric %	Gravimetric %	Electrometric %
1	46.10	46.19	99.60	99.69
2	45.90	46.37	99.30	99.77
3	46.20	46.13	100.04	99.97
4	46.40	46.34	99.70	99.64
5	46.10	46.13	99.90	99.93
6	46.10	46.25	99.90	100.05

Bureau of Standards dolomite sample 88 was dissolved in hydrochloric acid and the magnesium content determined potentiometrically. Values of 21.55 and 21.50% magnesia were obtained. This checks well against the Bureau of Standards value of 21.48% magnesia.

Most precipitated calcium carbonates contain quantities of magnesia which would lead to high results. Therefore, it is extremely important that analytical reagent calcium carbonate be used in this procedure. Some magnesite samples dissolve slowly in hydrochloric acid; consequently it has been found best in the laboratory to dehydrate the sample with perchloric acid. Better results are obtained by titrating the sample near its boiling point in a 50% solution of alcohol. These conditions lend sharpness to the end point. Thymolphthalein indicator assists in warning of the nearness of the end point, although it is in no way essential. A few milliliters of standard caustic are added to the sample before adjusting the instrument for the titration, since a considerable change in potential occurs with the first addition of caustic. A Beckman high-temperature glass electrode, No. 8990T, is recommended.

## SUMMARY

Magnesia in dolomite and magnesite may be determined directly by potentiometric titration in a hot 50% solution of alcohol. A procedure to eliminate the interference of weak bases such as ferric iron, aluminum, and titanium is described. The determination is rapid and simple, requiring but a few minutes for a single sample. The accuracy attained is comparable to that of the gravimetric ammonium phosphate method for magnesia.

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# Determination of Carbon Dioxide in Water

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Improved method for determining total carbon dioxide in water capable of a precision of  $\pm 1$  p.p.m. even in the presence of large amounts of interfering substances such as phosphates. It is believed that this is the best precision that can be obtained using open flasks for the titration. The method uses apparatus readily available in any laboratory, and requires no knowledge of the nature or concentration of interfering substances, except sulfides.

DURING the years 1931 to 1933, a number of papers (2, 3, 5, 7, 8, 9) were published on the determination and interpretation of alkalinity in boiler waters. This work led to the acceptance of the evolution method of Partridge and Schroeder for carbon dioxide as a tentative standard by the American Society for Testing Materials (1). The authors' experience indicates that this is the most precise method for determining carbon dioxide, but it suffers as a plant method from the fact that it is rather slow and requires special apparatus that is not easily portable nor always available. This, and the realization by steam plant operators that carbon dioxide may cause serious corrosion on condensate return lines, indicate the need for a rapid, accurate titration method requiring only apparatus readily available in any laboratory.

With the exception of the evolution method (1), titration methods, especially when used to determine small amounts of carbon dioxide (8), are defective in one or more of the following respects: (1) Indicators used do not change color at optimum pH values. (2) Titration is carried out by observing color change, and not to precise pH values. (3) Corrections due to the presence of interfering substances are uncertain.

The method described overcomes these defects by titrating the sample between two properly selected end points, acidifying and boiling off the carbon dioxide, cooling the sample, and retitrating between the same two end points. The difference between the two titrations gives the carbon dioxide in the sample. Titration between two end points is necessary to ensure the same effect of the interfering substances for the titration before and after boiling.

## SELECTION OF END POINTS

Titration curves were calculated as described by Schroeder (8) for carbon dioxide ranging from 4.4 to 440 p.p.m. and for a mixture of 44 p.p.m. of carbon dioxide with 95 p.p.m. of phosphate, using the values of the dissociation constants for carbon dioxide and phosphoric acid selected by Latimer (4). Inflection points (most rapid change of pH per unit of titrant) for the bicarbonate stage in the titration were very close to pH 8.5 in the absence of phosphate. The corresponding inflection point for the carbon dioxide-phosphate mixture occurred at pH 8.8. Inflection points for the free carbon dioxide stage shifted from pH 5 at the lowest carbon dioxide concentration to pH 4 at the highest, and were practically unaffected by phosphate. Since high precision is desired for small amounts of carbon dioxide, pH values 8.5 and 5 were selected. pH values 9 and 5 are slightly better in water containing phosphates.

In order to check the selection of pH 8.5 and 5.0 as end points, a number of samples were titrated using thymol blue and bromocresol green as indicators, with the results shown in Table I. Carbon dioxide was added as sodium carbonate and phosphate as potassium monohydrogen phosphate, with other substances as shown. End points were determined by comparison with LaMotte colorimetric standards. Titrations were conducted in open flasks, but as rapidly as possible to avoid contamination from the air. The sample volume was 100 ml. In all samples except No. 11, the agreement between the known carbon dioxide in the water and that found by analysis is within  $\pm 1$  p.p.m. A few samples were titrated using a glass electrode for the end-point determination. Repeated titrations differed by as much as 1 p.p.m. It is therefore believed that greater precision than  $\pm 1$  p.p.m. cannot be obtained in open flasks, because of chance loss of carbon dioxide to or gain from the atmosphere.

Errors caused by improper sampling and handling of the sample for analysis may greatly exceed the errors indicated above. Water samples for carbon dioxide should be analyzed promptly, and should be transferred from one vessel to another by siphoning rather than by pouring. The siphon should be immersed well below the water surface in the vessel being sampled and should deliver the sample to the bottom of the receiving vessel.

## SELECTION OF INDICATORS

Because of the blue alkaline color of both thymol blue and bromocresol green, they cannot be used together in the same sample. Each determination of Table I required the titration of four samples. A search was therefore made for indicators that could be used together in the same sample, and that were sufficiently stable to resist the necessary boiling, in order that a determination could be made on a single sample. Considering only indicators showing intense color contrast, methyl red, bromocresol green, and methyl orange were selected for the pH range 4 to 5, and thymol blue, phenolphthalein, and *o*-cresolphthalein for the pH range 8 to 9. Trials of pairs of these indicators led to the selection of methyl red mixed with *o*-cresolphthalein as the best pair, with methyl red mixed with phenolphthalein as a close second choice.

Table I. Results of Titration from pH 8.5 to pH 5

(Using thymol blue and bromocresol green indicators)

Known CO <sub>2</sub> in Water P.p.m.	Known PO <sub>4</sub> in Water P.p.m.	Other Substances in Water P.p.m.	Apparent CO <sub>2</sub> Unboiled sample, A P.p.m.	Boiled sample, B P.p.m.	Net CO <sub>2</sub> P.p.m.	Error P.p.m.
51	47.5	.....	80.1	30.4	50.7	-0.3
25.5	23.75	.....	40.2	14.9	25.3	-0.2
11.0	47	.....	40.3	29.7	10.6	-0.4
5.5	33	.....	20.5	16.4	4.1	-1.1
2.75	16.5	.....	11.5	9.3	2.2	-0.55
5.0	47.5	71 Na <sub>2</sub> SO <sub>4</sub> 10 Oxalic acid 10 Citric acid	37.5	31.6	5.9	+0.9
6.0	47.5	71 Na <sub>2</sub> SO <sub>4</sub> 10 Oxalic acid 10 Citric acid	37.8	31.2	6.6	+0.6
6.6	47.5	71 Na <sub>2</sub> SO <sub>4</sub> 10 Oxalic acid 10 Citric acid	37.2	31.7	5.5	-1.1
6.6	47.5	71 Na <sub>2</sub> SO <sub>4</sub> 10 Oxalic acid 10 Citric acid	37.2	30.4	6.8	+0.2
11.0	...	50 Na <sub>2</sub> SO <sub>4</sub>	10.3	0.0	10.3	-0.7
516.5	...	Contamination not known	540.0	21.2	518.8	+2.3
7.25	...	10 ml. of 0.02 N NaOH 209 p.p.m. NaNH <sub>4</sub> HPO <sub>4</sub> ·4H <sub>2</sub> O	70.0	63.3	6.7	-0.55
44.3	6.0	2 NaAlO <sub>2</sub> 30 Na <sub>2</sub> SO <sub>3</sub> 12 NaCl	57.7	12.4	45.3	+1.0

Figures in column A and B corrected for the amount of acid necessary to change pure water from pH 8.5 to pH 5.0, equivalent to 0.6 p.p.m. of CO<sub>2</sub>.



## REAGENTS REQUIRED

**MIXED INDICATOR SOLUTION.** Dissolve 0.1 gram of methyl red and 0.1 gram of *o*-cresolphthalein in 200 ml. of 50% alcohol (phenolphthalein may replace *o*-cresolphthalein).

**BUFFER SOLUTION.** Prepare buffer solutions for pH 8.5 and 5.0. If preferred, a pH 9.0 buffer may replace pH 8.5 buffer.

**ACID.** Hydrochloric or sulfuric acid, 0.02 *N*.

**BASE.** Approximately 0.02 *N* sodium hydroxide, carbonate-free, prepared by diluting a clear, saturated solution of sodium hydroxide with well-boiled distilled water, is satisfactory. Keep in a heavily waxed bottle, protected from atmospheric contamination by a guard tube of Ascarite or soda lime.

## PROCEDURE

1. Select two flasks of the size and type to be used in the titration (250-ml. Erlenmeyer flasks are satisfactory). Place 100 ml. of pH 8.5 buffer in one and 100 ml. of pH 5.0 buffer in the other, to each add 0.4 ml. of mixed methyl red-*o*-cresolphthalein indicator, and cork the flasks. After some practice, more or less indicator may be preferred, but the volume used in the buffered solutions should always be the same as that used in the sample.

2. In a third flask, add 0.4 ml. of mixed indicator to 100 ml. of the water to be tested.

3. Titrate to pH 8.5 (matching the buffered solution), using 0.02 *N* hydrochloric acid if the sample is more alkaline than pH 8.5 or 0.02 *N* sodium hydroxide if it is more acid. If sodium hydroxide is used, record the volume required as  $V_{1X}$  (see below, interfering substances in sodium hydroxide solution).

4. Titrate from pH 8.5 to pH 5 (again matching the buffered solution) with 0.02 *N* hydrochloric acid. Record the volume used as  $V_1$ .

5. Acidify by adding 20% more of the 0.02 *N* hydrochloric acid than was required for  $V_1$ , but in no case less than 5 drops of this solution. Boil the solution vigorously for 2 minutes over a strong flame.

6. Cool the flask rapidly in running water to room temperature, add 0.02 *N* sodium hydroxide until the pH is 8.5, and record the volume of base required as  $V_{2X}$ . Now titrate the sample from pH 8.5 to pH 5 with 0.02 *N* hydrochloric acid and record the volume of acid used as  $V_2$ .

## INTERFERING SUBSTANCES IN SODIUM HYDROXIDE SOLUTION

The sodium hydroxide solution is used only to adjust the pH of the sample to 8.5; hence, its exact normality need not be known. However, it may contain appreciable quantities of carbon dioxide, which would be titrated by the acid, and for which corrections must be made. The magnitude of the correction is determined as follows:

To 80 ml. of carbon dioxide-free distilled water add 0.4 ml. of mixed indicator and sufficient 0.02 *N* sodium hydroxide to bring the pH to 8.5. Then titrate to pH 5 using 0.02 *N* hydrochloric acid. Let the volume of acid used be *A*. Add immediately 20 ml. of 0.02 *N* sodium hydroxide and again titrate with 0.02 *N* hydrochloric acid, noting the volume required to change the pH from 8.5 to 5. Let this volume be *B*. Disregard the volume of acid required to titrate the 20 ml. of base to pH 8.5. The volume of acid required to titrate the carbon dioxide in 1 ml. of base is then:

$$\frac{B - A}{20} = X$$

## CALCULATION OF CARBON DIOXIDE

The carbon dioxide in the sample is calculated by the following formula:

$$\text{CO}_2 \text{ (p.p.m.)} = K \times \frac{1000}{V_s} \times N [(V_1 - V_{1X} \times X) - (V_2 - V_{2X} \times X)]$$

where  $K$  = 45.56 if the titration from pH 8.5 to 5 is used

or  $K$  = 43.95 if the titration from pH 9 to 5 is used

$V_s$  = sample volume in ml. (usually 100 ml.)

$N$  = normality of HCl solution

$V_1, V_2$  = ml. of acid required to titrate, respectively, un-boiled and boiled sample from pH 8.5 to 5 (or pH 9 to 5 if these end points are used)

$V_{1X}, V_{2X}$  = ml. of base used to adjust unboiled and boiled sample, respectively, to pH 8.5 (or 9)

$X$  = ml. of acid required to titrate  $\text{CO}_2$  in 1 ml. of base from pH 8.5 to 5 (pH 9 to 5)

The numerical value of  $K$  differs slightly from 44.01 (the molecular weight of carbon dioxide) since not exactly one equivalent of carbon dioxide is titrated between the pH values chosen (3, 5). To calculate  $K$  for a pH range other than those given above, the later tables of McKinney (6) should be used.

In many water supplies, the concentration of interfering substances remains practically constant, as evidenced by the constancy of the titration on the sample after expulsion of the carbon dioxide by boiling. In such cases, titration of the boiled sample need only be run often enough to determine  $(V_2 - V_{2X} \times X)$  in the formula above. The time required to run a single complete test is approximately 10 minutes. When a series of tests is run, the average time per determination is approximately 5 minutes, since a second sample may be titrated while the first is boiling and cooling.

## ACID GASES OTHER THAN CARBON DIOXIDE

Water supplies may be encountered containing sulfur dioxide or hydrogen sulfide or their salts. In the pH range 8.5 to 5 sulfites are titrated from  $\text{SO}_3^{--}$  to  $\text{HSO}_3^-$  and sulfides are titrated from  $\text{HS}^-$  to  $\text{H}_2\text{S}$ . Sulfites, at the low concentrations used in industrial water treatment, do not interfere with the carbon dioxide determination, as is indicated by sample Table I. This is as expected, since the solubility of sulfur dioxide, corrected for ionization, is about 40 times that of carbon dioxide and in addition only approximately 5% of the sulfur dioxide is in the un-ionized form at pH 3 (the approximate pH at which the carbon dioxide is boiled off). The sulfur dioxide is therefore not boiled off and is properly accounted for by the titration after boiling.

The properties of hydrogen sulfide are similar to those of carbon dioxide. Its solubility is approximately 3 times that of carbon dioxide and at pH 3 it is practically all in molecular form. Hence, it will be boiled off with the carbon dioxide, thus causing high results by the present method. The results may be corrected for hydrogen sulfide by the following procedure.

Collect the distillate from step 5 of the procedure in cadmium chloride solution and determine hydrogen sulfide therein in the same manner as for sulfur in steel by the "evolution method." Correct the carbon dioxide (p.p.m.) calculated from the formula given above by subtracting

$$\text{H}_2\text{S (p.p.m.)} \times \frac{44.01}{34.08} \times 0.9800 = \text{H}_2\text{S (p.p.m.)} \times 1.266$$

if the titration from pH 9 to 5 is used, or

$$\text{H}_2\text{S (p.p.m.)} \times \frac{44.01}{34.08} \times 0.9618 = \text{H}_2\text{S (p.p.m.)} \times 1.242$$

if the titration from pH 8.5 to 5 is used.

Samples containing large quantities of either hydrogen sulfide or sulfur dioxide cannot be successfully titrated by the procedure described in this paper, because of rapid reduction of the metal on boiling.

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# Determination of Iron

## A Study of the o-Phenanthroline Method

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A critical study was made of the o-phenanthroline method. The order of color development was influenced by the order of, and time interval between, additions of reagents, temperature of solutions, and amount of phosphate, and the length of time the solutions stood before being read in the photometer. If the reaction was tested with sodium citrate instead of acetate and the citrate was added after the hydroquinone and o-phenanthroline at temperatures above 20° C., iron was completely recovered.

DURING some nutrition experiments, the iron content of the rations and of individual yolks and whites of eggs was determined by the o-phenanthroline method (2, 3, 4). In this method, ashed materials are dissolved in dilute hydrochloric acid. Sodium acetate is added to aliquots to adjust the pH to 3.5, hydroquinone to reduce the iron, and o-phenanthroline to develop an orange-pink color. Iron in the form of o-phenanthroline complex is evaluated by means of a photometer using a Corning 430 blue-green filter. Certain difficulties developed and a study of the method was therefore made.

### EXPERIMENTAL

On the addition of acetate to solutions of the ash of the materials frequently produced turbid solutions which could not be read directly in the photometer (a Cenco Sheard-Sanford Photometer was used). Other acetates besides sodium were tried with the same result. Cowling and Benne (1), working with egg ash, overcame this by adding ammonium citrate before the acetate was adjusted to 3.5 by acetate. It occurred to the authors that citrate might replace the acetate in adjusting the pH and eliminate the turbidity. To check this hypothesis, aliquots of an acid solution of the ash of a poultry ration were adjusted to pH 3.5 with sodium acetate, sodium citrate, or potassium citrate. The acetate gave a cloudy solution which required centrifugation, whereas the citrates were clear. All three, however, gave correct iron values. The rate of color development with sodium citrate was the same as with the acetate but it was somewhat slower with potassium citrate.

Since sodium citrate was more satisfactory than the acetate in adjusting the pH, the range in pH over which it could be used was still retained. Maximum color development was ascertained. Aliquots of ashed egg white, yolk, or poultry ration, 0.5 to 1.0 g. of a 25% sodium citrate solution were added and the pH and iron determined. Maximum color development occurred above pH 2.5 and it remained maximum to pH 5, which was as high as was obtained with the amounts of citrate used.

The final solutions stood either 30 or 60 minutes before reading in the photometer, the order of addition of reagents was found to be of importance. Whenever the solution used to adjust the reaction to pH 3.5 was added first, as in the Hummel and Willard procedure (3), or was added second, results were inconsistent (Table I).

If, however, both the hydroquinone and o-phenanthroline were added before the pH-adjusting solution, determinations checked well.

The relative order in which hydroquinone and o-phenanthroline were added was unimportant. If solutions stood for 120 minutes, the order of addition of reagents was not critical.

The time that elapsed between the addition of the acetate and other reagents was of no consequence when iron salts by themselves were used. With solutions of ashed egg yolk and white, however, this time interval was important; the longer the interval, the less iron was determined. To investigate the possibility that the phosphorus, which was present in the materials to an appreciable extent in proportion to the iron, might have caused these low results, ferrous sulfate and sodium pyrophosphate solutions were mixed, dried on the steam bath, and ashed overnight in the muffle. Only 34% of the iron was recovered. Other salts of phosphoric acid were then investigated in a similar manner. In Table II are given the data on the recovery of iron from solutions when these salts were present in an iron-phosphorus ratio similar to that of egg white. In general, if the adjusting solution, whether acetate or citrate, was added first, poor recoveries were obtained; these were even worse with ashed samples. When the acetate or citrate was added last, recoveries were complete in the case of unashed but not ashed materials.

A possible explanation for the failure to determine the iron in the unashed solutions when the pH-adjusting solution was added first is that an iron-phosphate complex is formed which does not react with the o-phenanthroline. In the ashed samples, it is probable that an insoluble iron meta- or pyrophosphate is formed which does not dissolve in the dilute hydrochloric acid.

Cowling and Benne (1) reported that if solutions of ferrous sulfate and sodium pyrophosphate were heated in a water bath

Table I. Effect of Order of Addition of Reagents upon Iron Determination in Solutions of Ashed Egg Yolk, Egg White, and Poultry Ration

Order of Addition of Reagents			Egg Yolk				Egg White, Citrate		Poultry Ration, Citrate	
			Acetate 30 min.	60 min.	Citrate 30 min.	120 min.	30 min.	120 min.	30 min.	120 min.
1st	2nd	3rd	Per cent recovery of iron							
A	HQ	o-P	77	84	75	100	81	95	92	100
A	o-P	HQ	83	89	74	100	91	96	89	100
HQ	A	o-P	99	99	78	100	91	100	94	100
o-P	A	HQ	100	99	82	100	83	100	95	100
HQ	o-P	A	100	100	100	100	100	100	100	100
o-P	HQ	A	100	100	100	100	100	100	100	100

A = solution used to adjust to pH 3.5.

HQ = hydroquinone.

o-P = o-phenanthroline.

Table II. Effect of Phosphates upon Recovery of Iron from Ferrous Sulfate Solution

(Fe:P = 1:100, read after 30 minutes)

Phosphate	Sodium Acetate Added				Sodium Citrate Added			
	First, Unashed	Last, Unashed	First, Ashed	Last, Ashed	First, Unashed	Last, Unashed	First, Ashed	Last, Ashed
None	100	100	100	100	100	100	98	100
CaH <sub>4</sub> (PO <sub>4</sub> ) <sub>2</sub> ·H <sub>2</sub> O	88	100	80	100	90	100	91	92
CaHPO <sub>4</sub> ·2H <sub>2</sub> O	96	100	48	92	88	100	96	98
(NH <sub>4</sub> ) <sub>2</sub> H <sub>2</sub> PO <sub>4</sub>	80	100	48	76	86	100	83	85
(NH <sub>4</sub> ) <sub>2</sub> HPO <sub>4</sub>	72	100	40	52	88	100	76	82
KH <sub>2</sub> PO <sub>4</sub>	100	100	60	88	88	100	96	97
K <sub>2</sub> HPO <sub>4</sub>	72	100	12	84	86	100	67	88
Na <sub>2</sub> HPO <sub>4</sub> ·H <sub>2</sub> O	80	100	24	80	83	100	79	80
Na <sub>2</sub> HPO <sub>4</sub> ·12H <sub>2</sub> O	72	100	20	100	86	100	52	74
Na <sub>2</sub> PO <sub>4</sub> ·12H <sub>2</sub> O	48	100	72	100	90	100	71	95
Na <sub>4</sub> P <sub>2</sub> O <sub>7</sub>	...	...	...	...	88	100	52	80
NaNH <sub>4</sub> HPO <sub>4</sub> ·4H <sub>2</sub> O	...	...	...	...	90	100	55	69
H <sub>3</sub> PO <sub>4</sub>	...	...	...	...	88	100	52	67



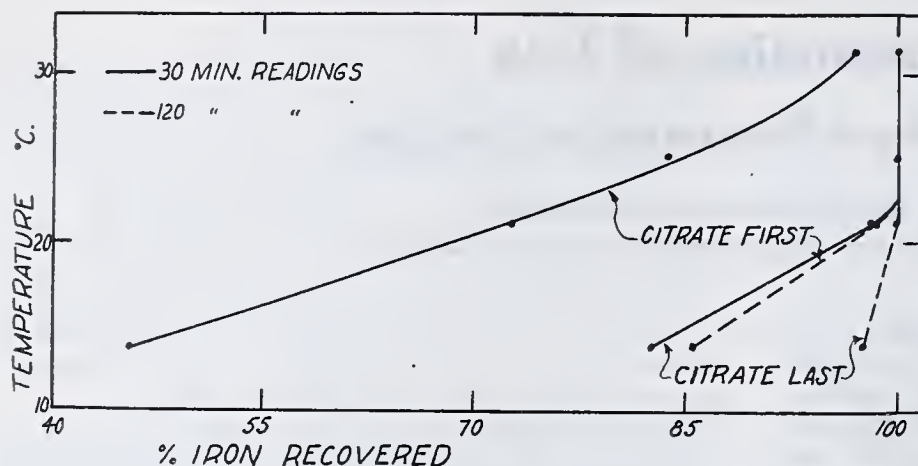


Figure 1. Effect of Temperature and Time of Standing upon Recovery of Iron from Egg White Ash  
Citrate added either first or last

for one hour after the addition of the reagents, color developed completely in the presence of considerable phosphorus as pyrophosphate. But they added the acetate before the other reagents to unashed substances. The authors show in Table II that, if either acetate or citrate is added last to unashed materials, heating is unnecessary. In an additional experiment, it was determined that, with solutions of ashed materials, heating as long as 60 minutes on a steam bath did not produce maximum color when the pH-adjusting solution was added first; furthermore, the longer the interval between the addition of this solution and the other reagents, the less the color developed.

The effect of temperature of the solutions before mixing was also investigated (Figure 1). At 14° C., less than half of the iron was determined in egg white ash when the citrate was added first and over 80% when the citrate was added last. At 21° and 25° C., about 75 and 85%, respectively, were found if the citrate was added first, whereas complete recoveries were obtained if the citrate was added last. At 31° C., practically complete recoveries were obtained no matter whether the citrate was added first or last. These data were obtained from readings after the solutions stood 30 minutes. If the solutions stood 120 minutes, the order of addition of reagents was immaterial except at 14° C. Similar results were obtained with egg yolk and a poultry ration. From this it is apparent that the temperature of the room or of the solutions is an important factor in the conduct of the procedure, particularly if the adjusting solution is added first.

To find the relative effect of temperature and the amount of citrate necessary for proper pH upon the rate of color development, varying quantities of 1 to 4 hydrochloric acid were added to aliquots of a solution of an ash from a poultry ration. These aliquots were adjusted to pH 3.5 with sodium citrate and the color was developed with hydroquinone and *o*-phenanthroline. Four different temperatures were used. If readings were made after 30 minutes, at any one temperature, the greater the amounts of citrate, the less the percentage of iron determined (Figure 2). For any given concentration of citrate, the lower the temperature, the less the percentage of iron determined. In other words, those solutions that required large amounts of citrate to produce a pH of 3.5 also required a higher temperature to produce maximum color. When the solutions stood 120 minutes, except at 14° C., neither the temperature nor volume of citrate affected the recovery of iron. If the citrate is added last, these complications do not arise.

Inasmuch as individual titrations to determine the amount of citrate required to adjust reaction to pH 3.5 slow up the procedure considerably, it was decided to find out if an average volume of citrate could be used when samples of similar materials of about the same size analyzed. A number of samples of egg yolk approximately the same weight were ashed, dissolved in acid, and made up to the same volume. Two aliquots of each solution were analyzed for iron. To the first was added the amount of citrate required to give pH 3.5 as determined by titration; to the second, the approximate average of the volumes of citrate required for the first aliquots. Good agreement between the two procedures was attained (Table III). The volume of citrate required for similar materials can be found by titrating about a dozen samples and using the whole number of milliliters just greater than the average of these values.

This can be done because the maximum color develops over a fairly wide pH range. Much labor, therefore, can be saved if an average volume of citrate is used instead of the varying volumes found by titrations when approximately equal samples of materials are being analyzed.

The procedure as outlined below is simple and has been found satisfactory by the authors.

#### REAGENTS

Iron Standard Solution (1 mg. of iron per ml.). Dissolve 0.1 gram of electrolytic iron in 50 ml. of 10% sulfuric acid and dilute to 1 liter with distilled water.

Table III. Comparison of Amounts of Citrate Obtained by Individual Titrations with Average of These Titrations

(Based upon determination of iron in a series of egg yolk samples)

Yolk Grams	Citrate Required Ml.	Individual titrations	Average of titration
		$\gamma$	$\gamma$
3.39	5.7	40.5	41.0
3.41	6.0	39.5	39.2
3.45	5.4	33.5	33.7
3.50	5.2	33.0	32.5
3.51	5.5	35.5	34.7
3.51	4.7	43.7	44.0
3.53	6.0	36.7	37.2
3.56	4.6	34.0	33.5
3.58	5.4	48.0	47.5
3.59	5.6	51.2	51.0
3.67	4.9	40.7	41.0
3.81	5.1	45.0	45.2
Av. 5.34		40.1	40.1

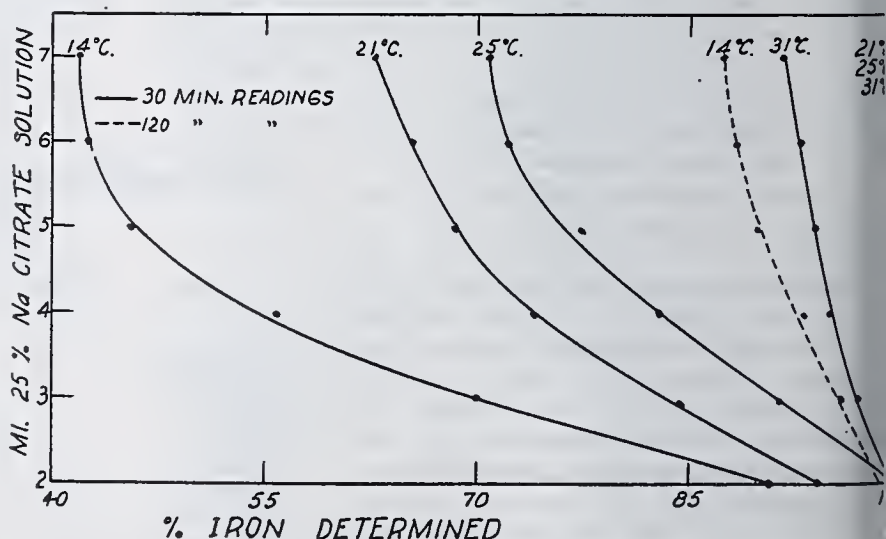


Figure 2. Effect of Temperature and Amount of Citrate upon Iron Determination



**Sodium Citrate Solution.** Dissolve 250 grams of sodium citrate in distilled water and make up to 1 liter.

**Hydroquinone Solution.** Dissolve 1 gram of hydroquinone in 100 ml. of distilled water. Store in refrigerator and discard if any color develops.

***o*-Phenanthroline Solution.** Add 150 ml. of almost boiling distilled water to 0.5 gram of *o*-phenanthroline in a 200-ml. volumetric flask. When cool, make up to volume. Store in refrigerator and discard if any color develops.

#### PROCEDURE

Pipet an aliquot of the unknown solution containing an iron concentration suitable for the range of the photometer into a 10-ml. volumetric flask, add 1 ml. of the hydroquinone, 2 ml. of the *o*-phenanthroline, and the proper amount of the citrate solution, and make up to volume. Let stand 30 minutes at a temperature above 20° C. and read in the photometer, using a blank made from the reagents in the same way for the 100 setting (this eliminates correcting for iron in the reagents). Use 1-cm. absorption cells and a 12.5-mm. No. 430 dark-shade blue-green Wratten molded glass filter. Convert readings into concentrations of iron by referring to a curve made from the iron standard solution in exactly the same manner.

#### SUMMARY

Sodium citrate was found more satisfactory than the acetate in adjusting the reaction for the development of the color of the *o*-phenanthroline complex.

If the pH was adjusted before the introduction of *o*-phenanthroline, the rate of color development was influenced by such factors as the time interval between the addition of reagents, temperature of the solutions, type and amount of phosphate present, amount of citrate, and length of time the solutions stood before being read in the photometer. If the sodium citrate was added after the hydroquinone and *o*-phenanthroline at temperatures above 20° C., these factors did not adversely affect the recovery of iron. Under these conditions, maximum color developed when the solutions stood only 30 minutes.

For samples of similar materials of approximately the same size, it was found expedient to use an average volume of citrate rather than to titrate each sample individually.

The procedure for the *o*-phenanthroline determination of iron, modified as a result of the study is presented.

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## Furfural Determination Iodine Method for Hydrolyzed Wood Liquors

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A rapid method has been developed for the determination of furfural. It is based on the oxidation of furfural to furoic acid by iodine in alkaline solution, and with aqueous solutions of pure furfural shows excellent precision and is highly accurate. With samples that contain other iodine-consuming constituents in addition to furfural slightly high results are obtained, but the precision is satisfactory. The method is well suited for control work in which accuracy is second in importance to the rapidity with which determinations may be made.

BECAUSE of the large number of furfural analyses required for control work in the author's laboratory, a simple and rapid method with a reasonable degree of accuracy was desired. The rapid bisulfite method of Jolles (3) and the bromine method of Hughes-Acree (2), both volumetric, were not practical, owing to the nature of the samples and the specific control conditions required by the methods. The gravimetric phloroglucinol (1) and 2,4-dinitrophenylhydrazine (7) methods gave sufficiently accurate results, but were much too slow. The phloroglucinol precipitation method was found to be the most practical and was used until the development of the present procedure.

A slight excess of iodine in strongly alkaline solution reacts quantitatively with furfural. In an approximately 1 *N* sodium hydroxide solution the hypoiodite, which is formed from the iodine, oxidizes the furfural quantitatively to furoic acid. By a method based on this reaction most of the difficulties found with other methods have been eliminated.

Pure furfural in aqueous solution can be determined accurately by this reaction. However, furfural used by the author is obtained from hydrolyzed wood liquors and is present in aqueous solution with lower boiling constituents which are termed "heads". Owing to the presence of these heads in the furfural samples, it has been necessary to devise a method that would

compensate for their iodine consumption. Furfural in solution with the heads is determined by carrying out a blank reaction on each sample in slightly alkaline solution in which the iodine preferentially reacts with essentially all the heads or lower alcohols, aldehydes, etc., but with only 12.5% of the furfural present, the furfural being oxidized to furoic acid. Then by the regular sample reaction in a 1 *N* sodium hydroxide solution the iodine required by all the furfural and heads is found and thus the total furfural in the sample is calculated.

#### DEVELOPMENT OF METHOD

**REGULAR SAMPLE REACTION.** Pervier and Gortner (6) first tried the use of iodine in alkaline solution as a method for determining furfural. Although they gave very few details of their work, they reported that the results could not be duplicated. Later Kline and Acree (4) also tried iodine in alkaline solution for determining furfural according to their method for determining aldose sugars (5). They gave no details of their work and reported only that negative results were obtained. Although very little information on the work of the previous investigators is given in the literature, it seems that their lack of success was due mainly to use of too large furfural samples and too low an alkalinity.

In the present work it has been found that the oxidation of furfural to furoic acid by iodine in alkaline solution is a function of the alkalinity. Approximately 100 mg. of pure furfural is as large a sample as can be oxidized quantitatively, regardless of the alkali concentration. With samples of furfural much larger than this, the oxidation is not quantitative, apparently because of the rapid formation of iodate and iodide before the hypoiodite originally formed has a chance to react with all the furfural present.

Figure 1 shows the effect of alkalinity on the oxidation of furfural to furoic acid by iodine when the reaction is carried out for 20 minutes at room temperature in a reacting volume of



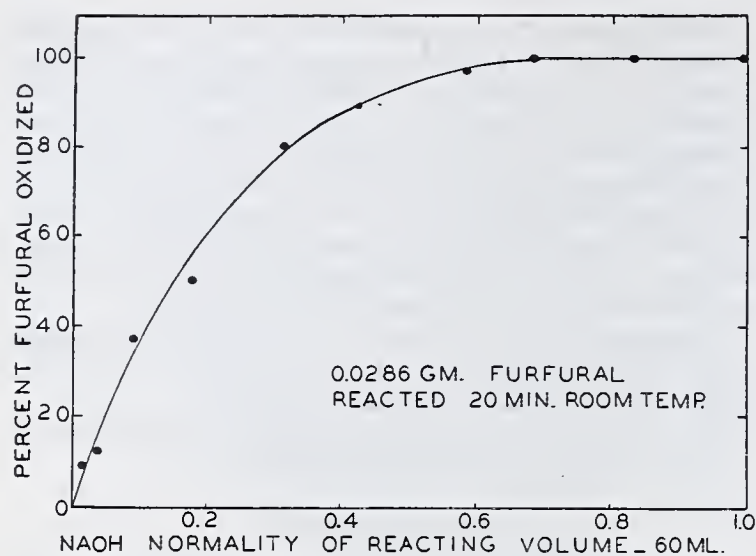


Figure 1. Effect of Alkalinity on Per Cent Furfural Oxidized

approximately 60 ml. Although the curve shows the oxidation to become quantitative when the alkalinity of the reaction mixture approaches 0.7 *N*, the conditions established for carrying out the reaction require an approximately 1 *N* sodium hydroxide solution to ensure complete oxidation. When the reaction was carried out in a 1 *N* sodium hydroxide solution a quantitative yield of furoic acid was isolated by extracting with ethyl ether and identified by melting point and mixed melting point with an authentic sample. The furfural used was redistilled and gave a purity of 100% by precipitating with phloroglucinol in 12% hydrochloric acid and by using Krober's tables.

Figure 2 gives the rate of oxidation of various concentrations of furfural when carried out at room temperature in a reacting volume of approximately 60 ml. of 1 *N* sodium hydroxide solution. The curves show that the oxidations are complete in 4 to 10 minutes, depending upon the size of the samples, and that additional time up to 30 minutes does not alter the reaction. If furfural samples as large as 100 mg. are used the time of the reaction should be increased to approximately 20 minutes to ensure complete oxidation. A slight increase in temperature does not affect the reaction other than to speed up the oxidation slightly.

**BLANK REACTION.** While the iodine required by the furfural is dependent upon the alkalinity, as shown in Figure 1, the iodine consumed by the heads is essentially independent of this factor. Conditions were established for the blank reaction whereby the heads consumed iodine to the same extent as they did in the regular sample reaction, and in which a consistent percentage of the furfural present was oxidized. By using 10 to 40 mg. of furfural in a reacting volume of approximately 60 ml. containing 10 ml. of 0.1 *N* iodine and the iodine equivalent of alkali or 2 ml. of *N* sodium hydroxide, 12 to 13% of the furfural is oxidized consistently. Furoic acid equivalent to an oxidation of 12.5% of the furfural has been isolated quantitatively from the reaction mixture and identified by melting points. The remaining 87.5% of furfural was shown to be left unchanged by accounting for it through precipitation with phloroglucinol in 12% hydrochloric acid.

Figure 3 gives the rate of the oxidation of furfural for various sized samples when this blank reaction is carried out at room temperature. From the curves it is seen that no further oxidation takes place after 18 to 20 minutes. A slight increase in temperature causes no change in the percentage of furfural oxidized, the only effect being to speed up the reaction slightly.

To determine the difference in the amount of iodine consumed by the heads in the blank and the sample reactions, a portion of the heads with a boiling range of 35° to 60° C. was fractionated from the crude furfural solution and 9 mg. reacted with the iodine

under each set of conditions. These low-boiling heads were used as they could be obtained free of furfural and constituted the major and most reactive portion of the impurities. Table I gives these results and shows a difference of only 0.1 ml. of 0.1 *N* iodine in the amount of iodine consumed by the heads in the blank and the sample reactions under the specific conditions outlined in the method.

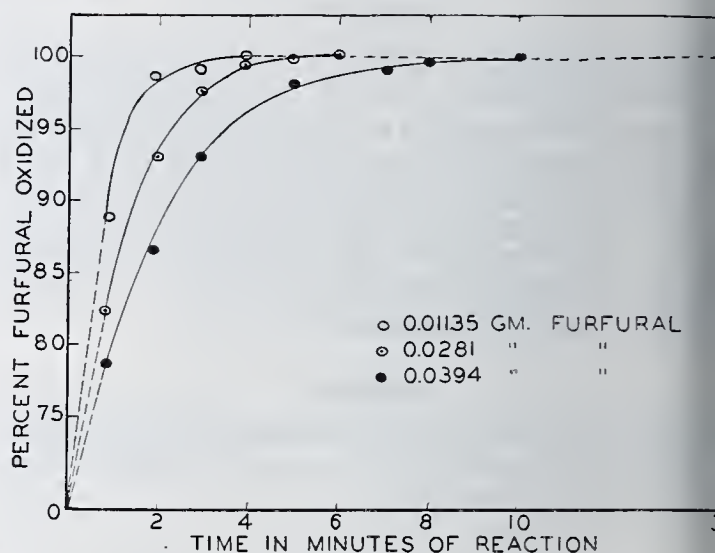


Figure 2. Rate of Sample Reaction at Room Temperature

After establishing the conditions for carrying out the blank reaction in which 12.5% of the furfural was oxidized and after showing from Table I that the iodine consumed by the impurities was essentially the same in both the blank and sample reactions, a comparison was made between the determination of furfural by this method and the phloroglucinol precipitation method. Samples were steam-distilled from their crude furfural solutions and the comparative results are given in Table II.

#### METHOD

Two aliquots of distillate containing 10 to 40 mg. of furfural are placed in 250-ml. wide-mouthed Erlenmeyer flasks. To these samples 4 or 5 drops of phenol red or any suitable aqueous indicator are added. One of the samples is made exactly neutral with sodium hydroxide and the other approximately so. The former is then employed as the blank and the latter as the regular sample.

Table I. Iodine Consumed by Heads (9-Mg. Sample)

Time Min.	0.1 <i>N</i> I <sub>2</sub> Consumed	
	Blank reaction Ml.	Sample reaction Ml.
5	1.10	1.20
10	1.15	1.45
15	1.25	1.45
20	1.35	1.45
30	1.35	1.45

Table II. Comparison of Iodine and Phloroglucinol Precipitation Methods

Sample No.	Per Cent Furfural	
	Phloroglucinol	Iodine
1	3.26	3.17
2	3.45	3.40
3	3.44	3.41
4	3.23	3.13
5	3.37	3.42
6	3.50	3.53
7	2.93	2.96
8	2.46	2.46
9	3.10	3.10
10	3.10	3.06
	Av. 3.18	3.16



to the blank, which is exactly neutral, are added 2 ml. of 1 *N* sodium hydroxide and then 10 ml. of 0.1 *N* iodine, the sample be swirled on addition of the iodine to ensure thorough mixing. After this addition the volume is adjusted to approximately 60 ml. with water and the solution allowed to stand at room temperature for 18 to 20 minutes.

To the regular sample, which was made approximately neutral, added 10 ml. of approximately 6 *N* sodium hydroxide. Immediately following this, 10 ml. of 0.1 *N* iodine are added and the solution is swirled during the addition. The volume is then adjusted to 60 ml. or approximately 1 *N* with respect to the sodium hydroxide and the solution allowed to stand at room temperature for 10 to 12 minutes. It is important that the sodium hydroxide be added before the iodine, since to obtain a quantitative oxidation the alkali concentration has to be rather high at the beginning of the reaction. Otherwise, the iodine is converted to the iodate and iodide by the strong alkali before the iodine initially formed has a chance to oxidize all the furfural quantitatively.

To the blank reaction, after standing 18 to 20 minutes, is added approximately 0.5 ml. of 6.5 *N* sulfuric acid. The excess iodine which is released from the iodate and iodide that has formed is titrated with 0.1 *N* sodium thiosulfate using starch indicator. The milliliters of thiosulfate are recorded as *B*.

Approximately 10 ml. or an excess of the 6.5 *N* sulfuric acid is added to the regular sample after it has stood 10 to 12 minutes. The excess iodine released is titrated with standard thiosulfate in the blank reaction. The milliliters of thiosulfate required are recorded as *S*. The value of *B* - *S* gives the milliliters of 0.1 *N* iodine required to oxidize 87.5% of the furfural present in sample to furoic acid.

Calculation: Grams of furfural in sample =  

$$\frac{(B - S) \times N \text{ of thiosulfate} \times 0.048}{0.875}$$

#### METHOD AS APPLIED TO DETERMINATION OF XYLOSE

To determine xylose by this method, by first converting the xylose to furfural with 12% hydrochloric acid, the regular Tollens distillation had to be modified.

Because the distillate has to be made neutral before the determination of furfural, only a 25-ml. aliquot of the acid distillate can be used, since the total reacting volume cannot exceed 60 ml. As it is necessary to use a larger sample of xylose than is called for in the Tollens distillation, so that the 25-ml. aliquot of the distillate will contain the appropriate amount of furfural. Xylose from the Eastman Kodak Company was used in this work and was of a purity of 100% when Krober's tables were used after the xylose had been converted to furfural by the regular Tollens distillation (1). In using the larger samples of xylose the following conditions were established for the conversion of the xylose to furfural in 12% hydrochloric acid.

From 0.6 to 1 gram of xylose is placed in a 1-liter balloon flask fitted with a dropping funnel and connected to a condenser by means of a Clark distilling head. Then 250 ml. of 12% hydro-

Table III. Conversion of Xylose to Furfural by Modified Tollens Method

Xylose Grams	Furfural Formed		Conversion Based on I <sub>2</sub> Method %
	Phloroglucinol method Gram	Iodine method Gram	
0.5990	0.330	0.324	84.4
0.8702	0.485	0.472	84.6
0.8350	0.450	0.445	83.2
0.9990	0.545	0.543	84.8
0.9948	0.542	0.540	84.7
0.9950	0.544	0.534	84.3
1.0004	0.552	0.544	85.0
			Av. 84.5

chloric acid are added to the xylose in the flask and distilled with an open flame at the rate of 50 ml. per 20 minutes, 50 ml. of 12% hydrochloric acid being added through the dropping funnel as 50 ml. distills over. In this manner 450 ml. of distillate are collected requiring a total time of 3 hours, the distillate is made to 500 ml., and 25-ml. aliquots are taken for furfural analysis according to method given.

By the use of the larger samples under the same conditions as employed above, the conversion of xylose to furfural is found to be 84.5% of the theoretical rather than the 88 to 89% found in the regular Tollens distillation. Thus the factor 0.54 is used to convert the furfural formed back to xylose instead of the Tollens factor of 0.57. Table III gives results for the conversion of pure xylose to furfural by this modified method.

#### SUMMARY AND DISCUSSION

A simple and rapid method for the determination of furfural has been developed. Furfural in pure aqueous solution can be determined accurately, by a quantitative oxidation, according to the regular sample reaction. In the presence of other iodine-consuming constituents, the method gives slightly high results but the precision is excellent. The results, even in the presence of these impurities, are slightly more accurate than with the phloroglucinol precipitation method which is considered a standard in most laboratories.

The interfering substances are in general those that will give the iodoform reaction at room temperature, although the blank reaction compensates almost quantitatively for these materials. No investigation has been made of the reaction between iodine in alkaline solution and methyl furfural or hydroxymethyl furfural. It is known, however, from the literature (8) that a method using iodine in alkaline solution is given as the basis for determining hydroxymethyl furfural in honey and there is little doubt but that iodine reacts with methyl furfural as well. The rapidity and excellent precision of the method have proved very satisfactory in this laboratory since its development 4 years ago.

#### ACKNOWLEDGMENT

The author wishes to thank the Masonite Corporation and especially Robert M. Boehm, director of research, for making it possible to publish this material.

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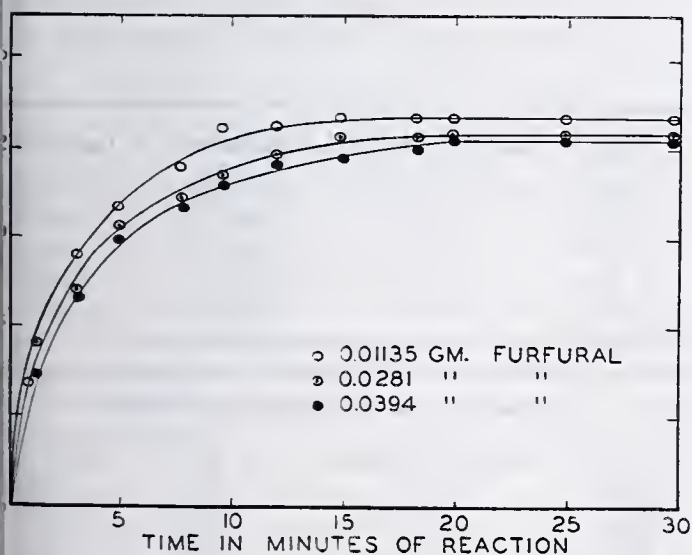


Figure 3. Rate of Blank Reaction at Room Temperature

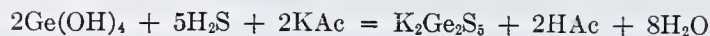


# Gravimetric and Volumetric Determination of Germanium

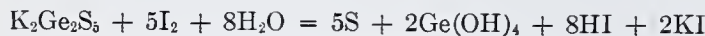
HOBART H. WILLARD AND C. W. ZUEHLKE<sup>1</sup>, University of Michigan, Ann Arbor, Mich.

A volumetric method for germanium was developed, based on the quantitative formation of potassium thiogermanate in acetate-buffered solution by treatment with potassium sulfide, removal of the excess hydrogen sulfide by carbon dioxide, and titration of the sulfur with iodine. The presence of large concentrations of sodium chloride leads to both a positive and a negative error in the application of this method, depending on conditions. The negative error is due to the adsorption of the sulfide ion on the free sulfur formed by titration with iodine, while the positive error is probably connected with the formation of a higher sulfide. Germanium is quantitatively precipitated by 5,6-benzoquinoline as a complex oxalate. Although the compound cannot be weighed directly, it can be ignited to germanium dioxide, thus affording a good gravimetric method. High concentrations of sodium chloride prevent this precipitation.

WILLARD and Zuehlke (4) recently reported the preparation of potassium thiogermanate. This compound serves as the basis of a volumetric method for germanium. Germanium dioxide, in acetate-buffered media, may be quantitatively converted to potassium thiogermanate by treatment with hydrogen sulfide or potassium sulfide:



The excess hydrogen sulfide is removed by passing a rapid stream of carbon dioxide through the solution, after which the sulfide ion is determined by treatment with an excess of standard iodine solution and back-titration with sodium thiosulfate solution:



## REAGENTS

Germanium dioxide solution, approximately 2 grams of germanium dioxide dissolved in 1 liter of water. This solution is stable and remains perfectly clear.

Acetic acid, 2.5 molar.

Potassium sulfide solution, approximately 8 grams of potassium hydroxide dissolved in 100 ml. of water and saturated with hydrogen sulfide. The solution was cooled to 0° C. during the saturation with hydrogen sulfide to avoid formation of thiosulfate and polysulfide by atmospheric oxidation.

Standard iodine solution, approximately 0.1 N.

Standard sodium thiosulfate solution, approximately 0.1 N.

## PROCEDURE

A measured aliquot of the germanium dioxide solution was transferred to a 25-cm. (10-inch) test tube and diluted to 25 ml., and 20 ml. of potassium sulfide solution were added, followed by 15 ml. of acetic acid solution. The acid was allowed to run down the side of the test tube to promote better mixing of the solution and to avoid a violent effervescence of hydrogen sulfide at the surface. The solution was allowed to stand for 5 minutes.

Carbon dioxide was bubbled through the solution rapidly by means of a delivery tube extending to the bottom of the test tube. A mechanical stirrer was used to break up the bubbles of carbon dioxide to promote more efficient removal of the excess hydrogen sulfide. Twenty minutes' passage of the gas was found to be sufficient to give a negative test for hydrogen sulfide in the issuing gases by lead acetate test paper.

The solution was transferred to a large beaker and diluted to 1 liter. A measured excess of standard iodine solution was added, allowed to stand for 15 minutes, and back-titrated with standard sodium thiosulfate solution using starch solution as an indicator. The end point may be determined to  $\pm 0.05$  ml.

## DISCUSSION

The success of the thiogermanate method depends upon stability of potassium thiogermanate in acid solution. It was previously reported that a solution of potassium thiogermanate contains insufficient free sulfide ion to precipitate cobalt or nickel sulfide. The carbon dioxide treatment readily removes the excess hydrogen sulfide from solution without decomposing thiogermanate. Further experiments were made by subjecting a solution of potassium thiogermanate to a rapid stream of carbon dioxide. Samples were withdrawn periodically and titrated with iodine to observe the decrease in sulfide-ion concentration with time. The results (Table I) show that the loss after 120 hours' treatment is negligible.

In order to attain complete reaction between hydrogen sulfide and germanium dioxide it is essential that a high concentration of sulfide ion be applied. The reaction is rapid but, nevertheless, is not complete when only the equilibrium concentration of a saturated solution of hydrogen sulfide is used. The data given in Table II were obtained by treating buffered solutions of germanium dioxide with a constant flow of hydrogen sulfide for varying periods of time. The excess hydrogen sulfide was removed and the solutions were titrated in the usual manner. Although the reaction is nearly complete after 5 minutes, quantitative completion is not attained even after 20 minutes.

The data in Table II indicate that the hydrogen sulfide-germanium dioxide system behaves as though an equilibrium is rapidly established a little short of complete reaction. Prolonged treatment with hydrogen sulfide is without beneficial effect but quantitative results are obtained when a condition of supersaturation is maintained for a short period of time by the addition of potassium sulfide.

Experiments concerned with the effect of temperature on the completeness of reaction are in agreement with this concept. Increasing the temperature decreases the solubility of hydrogen sulfide and, in general, decreases the amount of sulfur combined. Solutions of germanium dioxide buffered with acetic acid and potassium acetate were treated with hydrogen sulfide for 15 minutes at various temperatures. The amount of sulfur combined was determined in the usual manner. At 25° C. the reaction was 96% complete while at 80° C. only 65% of the germanium reacted in 15 minutes. In unbuffered solution only 11% reaction was obtained at these two temperatures, respectively.

The thiogermanate ion cannot be titrated directly with iodine to a satisfactory end point. Apparently the free sulfur formed

Table I. Stability of  $\text{K}_2\text{Ge}_2\text{S}_5$  Solution to  $\text{CO}_2$  Treatment

Time of $\text{CO}_2$ Passage Min.	0.1 N Iodine Solution Consumed Ml.	Time of $\text{CO}_2$ Passage Min.	0.1 N Iodine Solution Consumed Ml.
15	10.58	15	10.67 <sup>a</sup>
30	10.58	30	10.40
60	10.57	60	10.39
120	10.51	120	10.34

<sup>a</sup> Apparently some excess hydrogen sulfide still remained.

Table II. Rate of Formation of  $\text{K}_2\text{Ge}_2\text{S}_5$

$\text{GeO}_2$ Mg.	Time of $\text{H}_2\text{S}$ passage Min.	$\text{GeO}_2$ Found	
		At 25° C.	At 40° C.
48.6	5	47.9	47.7
48.6	10	48.1	48.1
48.6	15	48.3	48.5
48.6	20	48.2	

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Table III. Determination of Germanium by Thiogermanate Method

GeO <sub>2</sub> Taken Mg.	GeO <sub>2</sub> Found Mg.	GeO <sub>2</sub> Taken Mg.	GeO <sub>2</sub> Found Mg.
52.2	52.2	39.9	39.9
52.2	52.1	29.8	29.8
52.2	52.2	20.0	20.0
52.2	52.1	10.0	9.9
49.8	49.8		

The reaction adsorbs some of the thiogermanate ion and thereby prevents complete reaction and leads to a fading end point. The extent of this adsorption was shown to be dependent upon the concentration of electrolyte present. By dilution to a volume of liter before titration a true end point was obtained. This titration serves not only to reduce the concentration of electrolyte but also the concentration of the thiogermanate ion in equilibrium with the free sulfur as it is formed. The amount of iodine consumed when the equivalent of 50 mg. of germanium oxide was titrated in a volume of 250 ml. rather than 1 liter was found to be about 2% less than the true value.

This method is fairly simple, requires about 1.5 hours for a single determination, and is capable under favorable conditions of yielding precise results, as is shown in Table III.

#### EFFECT OF SODIUM CHLORIDE ON THIOGERMANATE METHOD

Germanium is usually separated for analysis by distillation as tetrachloride, or more probably, as a complex acid of the type  $H_2GeCl_6$ . Since this distillation is most efficiently carried out from constant-boiling hydrochloric acid, the final determination of germanium must frequently be made in a solution containing large amounts of chloride ion. It has already been shown that high concentrations of electrolyte lead to a false end point in the thiogermanate method. Accordingly an investigation was undertaken to determine the effect of sodium chloride on the application of this method. These experiments may be divided into three series:

1. Varying amounts of sodium chloride were added to the solution just prior to titration.
2. Varying amounts of sodium chloride were added to the solution prior to the sulfiding reaction.
3. Varying amounts of sodium chloride were added to the solution prior to the sulfiding reaction and the sulfur retained is determined by an inverse titration technique. In this procedure a solution containing an excess of iodine was diluted to about 800 ml. and the thiogermanate solution was added slowly with stirring. The sulfur was thereby formed in a solution containing very little unreacted thiogermanate ion and the sodium chloride was allowed to reach its highest concentration only at the end of the reaction. The results of these experiments are given in Table IV.

In considering the data collected in Table IV, except for a few irregularities, the following generalizations may be made. In the absence of sodium chloride the results are precise within the limits of experimental error. When sodium chloride is added just before titration a large negative error arises due to adsorption of the thiogermanate ion on the free sulfur formed. The magnitude of this error is proportional to the concentration of sodium chloride present and also the amount of germanium dioxide taken. When, however, the sodium chloride is present during the sulfiding process, the results are higher, apparently because of some kind of a compensating positive error. With small amounts of germanium dioxide this positive error predominates, leading to results which are above the theoretical. With large amounts of germanium dioxide the negative adsorption error predominates, leading to low results. When the inverted titration technique is used the results are generally higher than those obtained by the usual method, owing to the partial elimination of the negative adsorption error.

The low results in the sodium chloride solutions are unquestionably due to an adsorption error. The high results which arise when the sodium chloride is present during the sulfiding reaction are, on the contrary, not easily explained. This positive error may be connected in some manner with the formation of a higher sulfide of the type  $K_2Ge_2S_7$  (1), but not enough information concerning these thiogermanates is available at this time to arrive at any satisfactory conclusion.

The thiogermanate method, under favorable conditions, is capable of yielding results precise to 0.1 mg. of germanium, owing to its low equivalent weight in the thiogermanates (1 ml. of 0.1 *N* iodine solution is equivalent to 2.092 mg. of germanium dioxide, or to 1.452 mg. of elemental germanium). The scope of the method is seriously limited, however, by the sodium chloride error, since germanium is usually separated by distillation from constant-boiling hydrochloric acid. Undoubtedly many specific analytical situations do exist in which the method could be advantageously employed, particularly where the amount of germanium is low. Since germanium is frequently encountered as a minute constituent, the possibility of applying this method on the micro scale should not be overlooked.

Table IV. Effect of Sodium Chloride on Thiogermanate Method

GeO <sub>2</sub> Taken Mg.	No. NaCl added Mg.	GeO <sub>2</sub> Found			
		Grams of NaCl added	NaCl added after sulfiding Mg.	NaCl Added before Sulfiding Normal titration Mg.	Inverted titration Mg.
10.0	9.9	20	...	...	10.8
		30	9.5	9.8	10.8
		20	20.0	20.4	20.9
20.0	20.0	20	18.8	20.4	20.6
		30	29.3	29.7	29.6
		20	29.9	29.3	29.6
29.8	29.8	20	38.9	39.4	39.3
		30	37.9	38.8	38.8
		20	48.5	48.6	49.3
39.9	39.9	30	47.6	48.0	49.2
		20			
		30			
49.9	49.8	20			
		30			
		20			

#### INTERFERING SUBSTANCES

Metals which form insoluble sulfides at a pH of 4.6 must be absent.

#### GRAVIMETRIC METHOD

Willard and Toribara (3) showed that tin forms a complex oxalate and assigned the formula  $K_6Sn_2(C_2O_4)_7$  to its potassium salt. Tchakirian (2) attempted to prepare the analogous compound of germanium but found that the potassium salt was too unstable to isolate. He did, however, establish the existence of trioxalatogermanic acid,  $H_2Ge(C_2O_4)_3$ , by isolating it as the quinine and strychnine salts. It was found that 5,6-benzoquinoline also forms an insoluble derivative with trioxalatogermanic acid which shows promise as an analytical precipitant for germanium.

**REAGENTS.** Germanium dioxide solution, approximately 2 grams of germanium dioxide dissolved in 1 liter of water.

5,6-Benzoquinoline oxalate solution. Ten grams of 5,6-benzoquinoline (may be obtained from the Eastman Kodak Company) were treated with 5 grams of oxalic acid dissolved in 50 ml. of water. The suspension was heated to promote solution of the base, filtered while hot, and diluted to 500 ml.

**EXPERIMENTAL.** Measured aliquots of the germanium dioxide solution were diluted to 400 ml., treated with 5 grams of oxalic acid, and heated to promote the formation of trioxalatogermanic acid. Twenty-five milliliters of the reagent solution were added. Upon cooling to room temperature the derivative was precipitated in long crystalline needles. The solutions were allowed to stand overnight to ensure complete precipitation, and filtered. After washing with a dilute solution of oxalic acid and the reagent, the precipitate was ignited in a platinum crucible in a muffle at 700° to 800° C. to a pure white residue of germanium dioxide. Precipitation was found to be quantitative, as shown by Table V.



Table V. Precipitation of Germanium with 5,6-Benzoquinoline

GeO <sub>2</sub> Taken Mg.	GeO <sub>2</sub> Found Mg.	GeO <sub>2</sub> Taken Mg.	GeO <sub>2</sub> Found Mg.
84.3	84.2	49.9	49.9
54.2	54.0	49.9	50.1

5,6-Benzoquinoline would appear to be a superior precipitant for germanium for the following reasons: The precipitation procedure is simple and yields a product of very high molecular weight. The crystalline precipitate is easy to filter and wash and there is accordingly little danger of contamination by foreign ions. The precipitate is readily converted to germanium dioxide.

A specimen of 5,6-benzoquinoline trioxalatogermanate was purified by recrystallization from water. Weighed samples were ignited to the oxide and from the loss in weight a ratio of 1.98 moles of base for each mole of germanium was calculated. Because a definite composition was indicated, an attempt was made to precipitate the derivative in pure form and to weigh it directly. In all these attempts some of the reagent was coprecipitated, even when its concentration was reduced to the point where quantitative precipitation of the germanium was no longer obtained. The precipitate was also found to lose weight slowly in a desiccator and to come to a constant value only after 30 hours.

A concentration of 20 to 30 grams of sodium chloride in a vol-

ume of 400 ml. completely prevented precipitation of this derivative. It would appear that the germanium is firmly bound in a complex ion of the type  $\text{GeCl}_6^{--}$  under these conditions and therefore fails to form the oxalate complex which is necessary for precipitation.

Of the other members of the fourth periodic group, titanium, zirconium, and tin are known to form complex oxalates. 5,6-Benzoquinoline was found to give a precipitate when solutions of these elements were treated by the same procedure as was used for germanium. The precipitate formed with tin resembles that of germanium very closely, while the products obtained with titanium and zirconium appeared to be much more insoluble and flocculent in nature. These compounds are being investigated.

**INTERFERING SUBSTANCES.** All elements which form insoluble oxalates when treated with oxalic acid must be absent. Titanium, zirconium, tin, and to a lesser extent, iron, form complex oxalates which are precipitated by the reagent.

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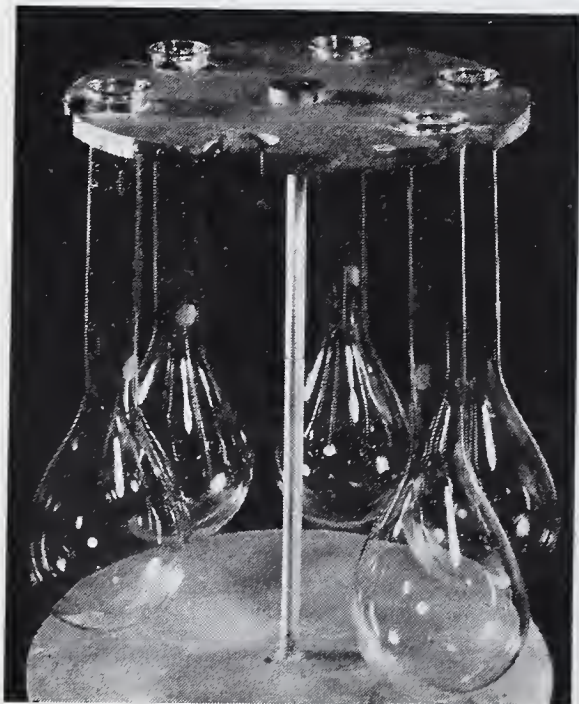
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From a dissertation submitted by C. W. Zuehlke to the Graduate School of the University of Michigan in partial fulfillment of the requirements for a degree of doctor of philosophy in chemistry.

## Support for Kjeldahl Flasks

JACQUELINE FRONT, Mellon Institute, Pittsburgh, Pa.

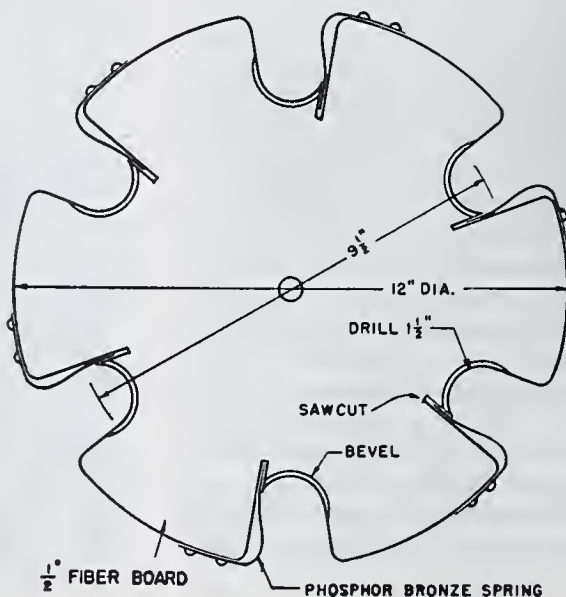
**B**ECAUSE of their peculiar shape, with round bottom and elongated wide neck, Kjeldahl flasks do not fit into any rack ordinarily found in a chemical laboratory. Tall rectangular supports have been used with clamps holding the flasks, but such arrangements are unstable and working the clamps is inconvenient and time-consuming. It is considered desirable to have a support which will grasp the flask, hold it firmly, yet release it with one movement. Furthermore, it should hold the flasks vertically, and provide easy access to each flask, as samples are measured into them.



The necks and lips of the flasks are not uniform in size; therefore, the holding device cannot be rigid if it is to accommodate these differences. To solve this problem in this laboratory, a holder of simple design has been made. The beveled opening of the flask is provided with a spring (see drawing). The fiberboard (plastic) disk has six places for flasks, although any number can be used. This disk is constructed so that it rotates on a central shaft and each flask is equally accessible. To stabilize the support, a heavy circular base of metal is employed, with a diameter of 35 cm. (14 inches) extending out as far as the widest part of the hanging flask.

The upper portion of the neck of the flask is pushed into the disk opening, so that, when the flask drops down, it catches on the bevel and is held firmly by the spring about 1.25 cm. (0.5 inch) below the lip. To remove a flask from the rack it is merely necessary to raise the flask straight up and slide it out.

This stand is very useful in holding the flasks when either solid or liquid samples are measured into them. It is substantial enough to support several 800-ml. flasks and the contents with no tendency to tip. Ammonia distillations and nitrogen digestions have been facilitated considerably by this inexpensive and easily constructed support.



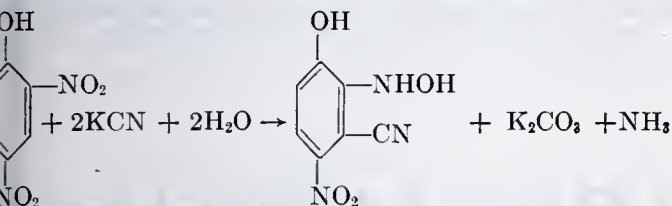


# Colorimetric Determination of 2,4-Dinitroanisole

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THE development of insecticidal dusts (2, 5) containing 2,4-dinitroanisole has raised the problem of a suitable method for the determination of this compound in insecticidal compositions. The presence of a yellow color in some preparations due to other materials, such as pyrethrum oleoresin, made it impractical to use the yellow color of 2,4-dinitrophenol produced by the action of alkalis on 2,4-dinitroanisole as the basis of a photometric method. Use was made of the purpuric acid reaction (3, 4), in which potassium cyanide reacts with *m*-dinitro compounds to give red-brown to violet colors. By analogy, the reaction with 2,4-dinitroanisole is presumed to be similar to that with 2,4-dinitrophenol, which is as follows:



The equation given by Anger (1) and reproduced by Feigl (4) is not balanced and could be made to balance only if potassium carbonate is a product rather than potassium bicarbonate, as shown by Feigl.]

Feigl (4) described this test for a number of *m*-dinitro compounds but did not list 2,4-dinitroanisole, which the authors found gave a red color with the potassium cyanide reagent. He did not list 1-chloro-2,4-dinitrobenzene, which sometimes occurs as an impurity in 2,4-dinitroanisole and would therefore interfere. However, a specific method for determining 1-chloro-2,4-dinitrobenzene in 2,4-dinitroanisole has been developed (6). In the case of 2,4-dinitroanisole (and 1-chloro-2,4-dinitrobenzene) heat was not found to be necessary for rapid development of the color, but does seem to be necessary for many of the other *m*-dinitro compounds, such as 2,4-dinitrophenol and dinitrocyclohexylphenol.

The following procedure was developed for an insecticidal dust containing 2% of 2,4-dinitroanisole, 2% of *N*-isobutyl unlenamide, enough pyrethrum oleoresin to give 0.2% of total pyrethrins, and 1% of an antioxidant, with pyrophyllite as the inert:

Weigh 2.000 grams of the powder into a small beaker. [For dusts containing 10% of 2,4-dinitroanisole, such as is recommended by Gould (5), about 0.400 gram suffices.] Stir the sample with four or five portions of acetone, decanting each time through a Gooch crucible that contains an asbestos mat and is placed in an all-glass Gooch funnel. With care, the solution may be filtered directly into a 100-ml. volumetric flask, if a large Gooch filtering bell jar is used. Continue to wash with acetone until the volume is nearly 100 ml. and then make up to volume with acetone. The solution should be perfectly clear and will have a yellow color due to the pyrethrum oleoresin and other constituents. Take a 10.00-ml. and a 15.00-ml. aliquot, and add 5.00 ml. of acetone to the 10.00-ml. aliquot. Prepare comparison standards containing 2, 4, and 6 mg. of pure 2,4-dinitroanisole diluted each to 15.00 ml. with acetone. These standards are conveniently prepared from a standard solution containing 40 mg. of 2,4-dinitroanisole per 100 ml. of acetone. Add 5.00 ml. of 5% aqueous potassium cyanide to each of the solutions, mix, and stand for one hour, and measure the color in a photometer, using acetone as the blank. An Aminco photometer, type F, with photometer test tubes and a No. 58 yellow filter (wave length of maximum transmission at about 580 millimicrons) is suitable. It was found by experiment that if a No. 58 filter is used there is no interference from the other constituents in the dusts in which they are present in the insecticide dust, even though they contribute a yellow color to the acetone solution.

The results for the standards may be plotted as per cent transmission against concentration on semilogarithmic paper, or as  $\log \left( \frac{100}{\% \text{ transmission}} \right)$  against concentration on ordinary graph paper. The concentration of 2,4-dinitroanisole in the unknown may then be read off this graph. The color follows Beer's law, as shown in Figure 1.

Acetone was used as the solvent in order to obtain a rapid and complete solution of the constituents of the powder without having to resort to a Soxhlet or other extractor. However, potassium cyanide is not soluble in acetone, and a 0.5% aqueous solution of this reagent had to be used. The amount of water thus added to the acetone solutions was found to be sufficient to keep the potassium cyanide in solution without precipitating any of the dissolved organic substances.

As an example of the precision to be expected, a commercial sample of dust supposed to contain 2% of 2,4-dinitroanisole plus

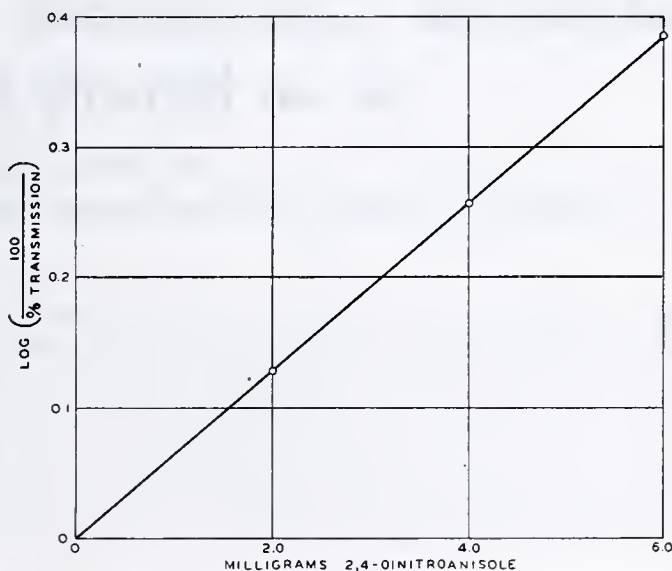


Figure 1. Determination of 2,4-Dinitroanisole Using Potassium Cyanide as the Reagent

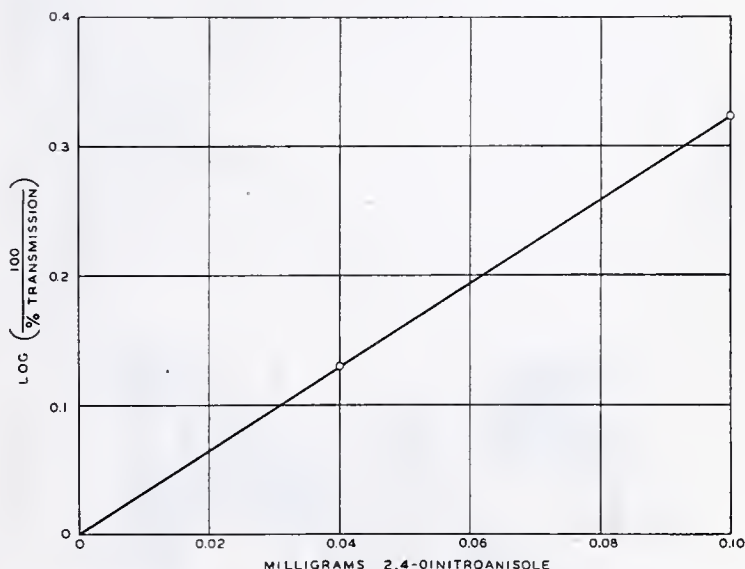


Figure 2. Determination of 2,4-Dinitroanisole Using Acetone and Alkali



the other constituents named above, when analyzed by the proposed method gave, as an average of 10 determinations, 2.17% with an average deviation of 0.05 and a maximum deviation of 0.12% when the 10-ml. aliquots were used, and 2.11% with an average deviation of 0.04 and a maximum deviation of 0.11% with the 15-ml. aliquots.

A more sensitive color reaction took place when 25 ml. of an acetone solution of 2,4-dinitroanisole were shaken with 5 ml. of concentrated sodium hydroxide solution (about 35%), with which it is immiscible. After the solution has been shaken for several minutes in a glass-stoppered cylinder and has stood for 30 minutes, a beautiful violet color develops in the acetone layer. The acetone may be decanted into a photometer tube and the color measured photometrically (a No. 58 filter on the Aminco photometer, type F, was suitable).

Some data to illustrate the sensitivity of the method are plotted in Figure 2. The test was found to be too sensitive to obtain concordant results in the analysis of dusts containing as much as 2% of 2,4-dinitroanisole, possibly because of the greater effect of interferences on such a sensitive reaction. However, this method would certainly be useful where very small amounts of

2,4-dinitroanisole had to be determined. This reaction is similar to that described for the analysis of *m*-dinitrobenzene (7). Chloro-2,4-dinitrobenzene, which sometimes occurs as an impurity in 2,4-dinitroanisole, also gives this color reaction. One of the interferences which must be scrupulously avoided is the presence of even traces of sulfur, such as contamination from the sulfur in rubber stoppers. Sulfur destroys the violet color, producing a greenish coloration instead.

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## Colorimetric Determination of 1-Chloro-2,4-dinitrobenzene as an Impurity in 2,4-Dinitroanisole

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ORDINARILY 2,4-dinitroanisole is manufactured either by the methylation of 2,4-dinitrophenol or by the reaction of alkali and methanol with 1-chloro-2,4-dinitrobenzene. In the latter process some of the chlorodinitrobenzene may be left in the final product, even after recrystallization. In fact, all commercial samples of 2,4-dinitroanisole made from 1-chloro-2,4-dinitrobenzene which have been examined by the authors, even those that were supposedly purified by recrystallization,

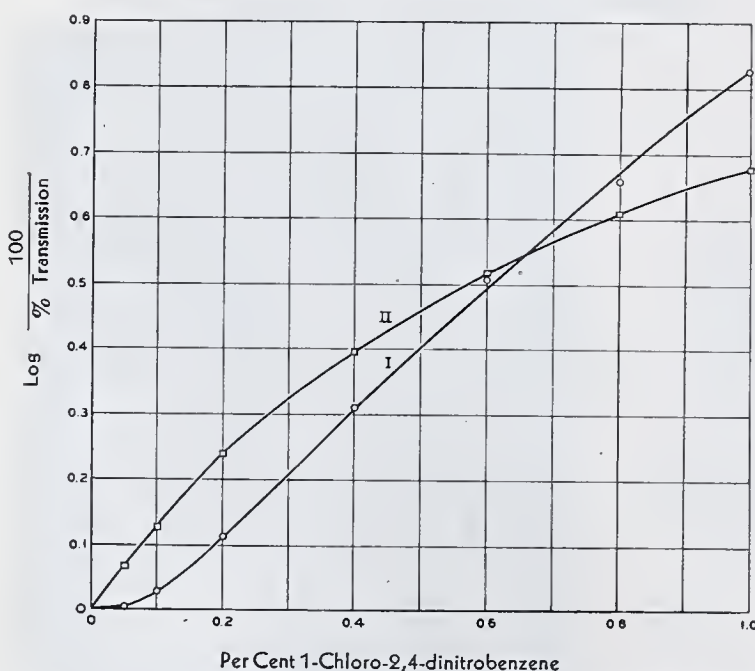
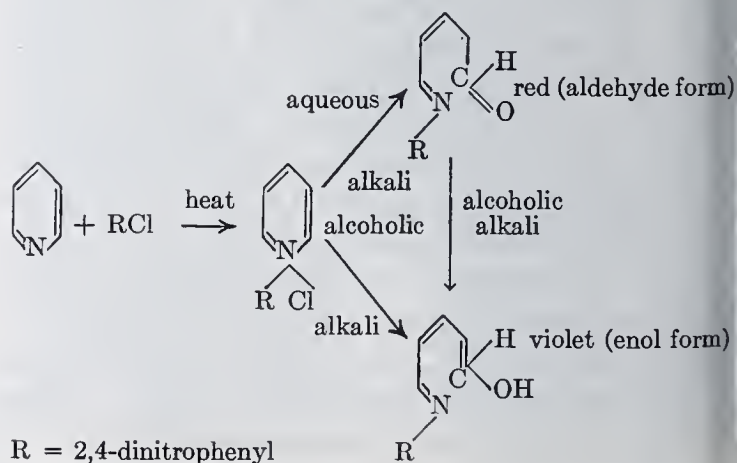


Figure 1. Determination of 1-Chloro-2,4-dinitrobenzene in 2,4-Dinitroanisole

when analyzed by the colorimetric method outlined below proved to contain about 0.5% of 1-chloro-2,4-dinitrobenzene. Procedures for determining 1-chloro-2,4-dinitrobenzene in 2,4-dinitroanisole based on the conversion of the chlorine to chloride by heating in alkaline solution and gravimetric or titrimetric determination of the chloride are unsuitable for small concentrations of the chlorodinitrobenzene, since a large sample has to be used and a correction also has to be made for inorganic chloride derived from salt left in the compound in the process of manufacture.

2,4-Dinitroanisole is used as an insecticide (1, 2), and a method for its analysis in insecticidal powders is described (4). Since 1-chloro-2,4-dinitrobenzene is a powerful skin irritant and repeated contacts may cause hypersensitization (5, 8), it is desirable to have a sensitive colorimetric method of analysis for this compound in dinitroanisole.

The procedure developed is based on the Vongerichten reaction (7, 9):





This reaction was first used in the detection of pyridine using 1-chloro-2,4-dinitrobenzene as a reagent (7) and has also been used recently in the determination of nicotinic acid and its salts (3, 6), but it has never been applied to the determination of 1-chloro-2,4-dinitrobenzene. It is very specific, since neither *o*-chloro-2,4-dinitrobenzene nor *p*-nitrochlorobenzene gives the reaction. Quinoline does not react and therefore cannot be used instead of pyridine.

Since commercial samples of 2,4-dinitroanisole made from 1-chloro-2,4-dinitrobenzene contain about 0.5% of the latter, the following procedure was developed for amounts of this order, although the method can be made more or less sensitive as desired by using different-sized samples or aliquots. Standards made from pure 2,4-dinitroanisole to which known amounts of 1-chloro-2,4-dinitrobenzene are added and heated with pyridine can be prepared, for two reasons: (1) The 2,4-dinitroanisole is partially converted to 2,4-dinitrophenol, thus contributing a yellow color to the solutions; and (2) the amount of color produced by heating 1-chloro-2,4-dinitrobenzene with pyridine in the presence of such 2,4-dinitroanisole is considerably less than when no 2,4-dinitroanisole is present. Pure 2,4-dinitroanisole used in the standards of comparison may be prepared by recrystallizing from ethanol the product prepared by the methylation of 2,4-dinitrophenol.

#### PROCEDURE

Place 0.500 gram of each sample in a photometer test tube that has been marked for a capacity of 25 ml. For standards of comparison, use pure 2,4-dinitroanisole (free of 1-chloro-2,4-dinitrobenzene) in the following amounts: 0.500, 0.500, 0.499, 0.498, 0.497, 0.496, and 0.495 gram. Weigh accurately 50 mg. of 1-chloro-2,4-dinitrobenzene into a 50-ml. volumetric flask. From a stock solution add 5.00 ml. of colorless pyridine to each of the samples to be analyzed, and to the standard comparison tubes add, in order, 5.00, 4.00, 3.00, 2.00, 1.00, and 0 ml. Immediately make up each 1-chloro-2,4-dinitrobenzene up to 50.00 ml. with pyridine, mix thoroughly, and add to the standard tubes, in order, 0, 0, 2.00, 3.00, 4.00, and 5.00 ml. of this standard solution. As this solution will darken in a short time, it should be made up fresh for each analysis and used immediately. Swirl each test tube until the solution is homogeneous, and heat all the tubes in a boiling-water bath for 30 minutes. Cool, make up to 25 ml. by adding ethanol, mix thoroughly by swirling, and measure the color in a photometer using a No. 58 color filter (one having its maximum length of maximum transmission at about 580 millimicrons; Minco photometer, type F, and appropriate photometer test tubes were used). The standards containing 0.500 gram of 2,4-dinitroanisole and no 1-chloro-2,4-dinitrobenzene are used as blanks to balance the photometer at 100% transmission. This is reading I. The red color is presumably due to the presence of compound A.

With a pipet place 1.00 ml. of each of the foregoing solutions in a set of photometer tubes, make up to 25 ml. with ethanol, add 1.00 ml. of colorless 2% ethanolic sodium hydroxide solution, mix thoroughly by swirling, stopper with corks, and measure the color of this set of solutions in the photometer using a No. 58 color filter. This gives reading II. The color is presumably due to the presence of compound B. Because of the yellow color of 2,4-dinitrophenol, the solutions appear red rather than the pure red given by compound B. This reaction with alkali is far more sensitive than the red color given by reading I, and therefore interference from colored impurities will be much less. If the concentration of 1-chloro-2,4-dinitrobenzene in 2,4-dinitroanisole is low, an aliquot greater than 1.00 ml. should be used; if it is very low, the standards of comparison should be prepared with appropriately smaller concentrations of 1-chloro-2,4-dinitrobenzene, and aliquots greater than 1.00 ml. may also be used to increase the sensitivity.

The results may be plotted on semilogarithmic paper as per cent transmission against per cent concentration of 1-chloro-2,4-dinitrobenzene or on ordinary graph paper as  $\log \left( \frac{100}{\% \text{ transmission}} \right)$  against per cent concentration. The curves are illustrated in Figure 1. The per cent of 1-chloro-2,4-dinitrobenzene in the sample may be read from the graph.

The concentration read from the standard curve using reading I usually agrees with that read from the second curve using

reading II. However, some commercial samples known to contain no 1-chloro-2,4-dinitrobenzene appeared to contain a small amount when reading I was used but gave 0% when reading II was used. Since this was probably due to the presence of a small amount of colored impurity, reading II should be relied on when the sample is suspected of containing any colored impurities, which usually can be detected by visual comparison of the color of the original sample with that of the pure 2,4-dinitroanisole. The use of both readings serves as a check and may indicate the presence of colored impurities (other than 1-chloro-2,4-dinitrobenzene). If desired, reading I may be omitted altogether and the samples may be heated with pyridine in ordinary Pyrex test tubes, followed by dilution to the 25-ml. mark, and the placing of 1-ml. aliquots in photometer test tubes for reading II. With some experience, the number of standards to be prepared may be cut down, especially where the approximate concentration of 1-chloro-2,4-dinitrobenzene is known, but the order of operations should be carried out exactly as described.

A qualitative test for 1-chloro-2,4-dinitrobenzene in 2,4-dinitroanisole has also been developed, which enables the characteristic violet color of compound B to be seen without serious interference from the yellow color of 2,4-dinitroanisole and 2,4-dinitrophenol.

Heat 0.5 gram of the material with 2 ml. of pyridine in a boiling-water or steam bath for 20 to 30 minutes. Cool, add water in small amounts until the 2,4-dinitroanisole begins to crystallize, and then dilute to about 30 ml. Cool in an ice bath, filter the precipitate, and wash with a little cold water. Make the filtrate up to a definite volume such as 100 ml. and take an aliquot or all of it for the next step, depending on the amount of 1-chloro-2,4-dinitrobenzene expected. Add 10 ml. of 10% sodium hydroxide solution and extract with 10 ml. of chloroform. Wash the chloroform with 50 ml. of 2% sodium carbonate solution in another separatory funnel, and then filter the chloroform layer through a funnel containing a plug of cotton. The chloroform will be colored more or less yellow, depending on the amount of 1-chloro-2,4-dinitrobenzene originally present, and this itself is a fairly good test. Extract the aqueous solutions in both funnels successively with another 10-ml. portion of chloroform and, if necessary, repeat until the chloroform washings are colorless. Evaporate the combined chloroform solutions to about 1 ml. on a water bath and remove the rest at room temperature by applying a vacuum. Dissolve the residue in 2 ml. of 1-butanol, add 2 ml. of 10% sodium hydroxide solution, shake, and observe the red-violet color produced in the 1-butanol layer if any 1-chloro-2,4-dinitrobenzene was present. This color is more stable if the solution is kept cold, but it gradually turns yellow. If a blank is run for comparison, 0.01 mg. of 1-chloro-2,4-dinitrobenzene can be detected in 0.5 gram of 2,4-dinitroanisole.

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# Effects of Beta-Carotene Isomerization on Its Absorption at 326 Millimicrons

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THE importance of the influence of the carotenoid pigments upon the absorption at 326  $m\mu$  is well known to workers who are attempting to estimate vitamin A concentrations by spectrophotometric methods. The usual procedure is to measure the absorption due to carotene at 450  $m\mu$  and use a certain percentage of this value as the correction in the ultraviolet for vitamin A determination. Considerable discrepancy exists in these correction values as reported in the literature. Gillam (4) reports a factor of 6.5%, Steenbock (1) 10%, McNicholas (5) 5%, and Peterson (8) values ranging from 5 to 8.3%.

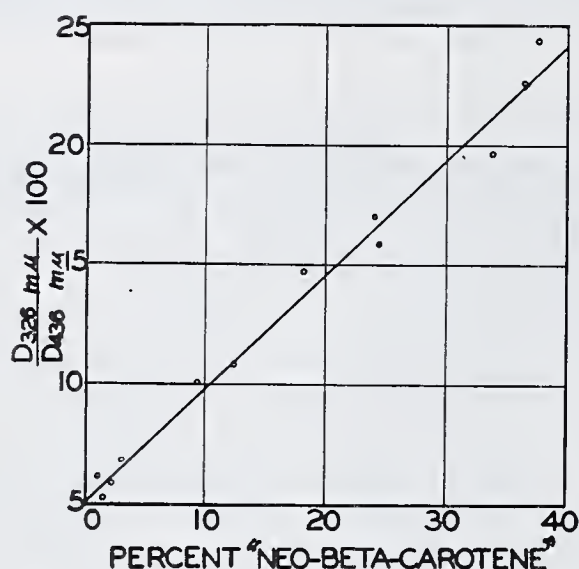


Figure 1. Relation between Optical Density Ratios and Calculated Per Cent Isomerization of  $\beta$ -Carotene

Recent studies on the carotenoids (2, 10) have shown that heat is one of the factors responsible for their isomerization. All the methods in general use for vitamin A determination require heating at some place in the procedure. It therefore appeared probable that isomerization phenomena were partially responsible for the range of correction values listed. Accordingly, a series of  $\beta$ -carotene extracts was analyzed spectrophotometrically at wave lengths of 436, 478, and 326  $m\mu$ , and the per cent isomerization to "neo- $\beta$ -carotene" was calculated by the method of Beadle and Zscheile (2). The ratios of optical densities at 326  $m\mu$  to those at 436  $m\mu$  were also calculated. (The optical

density is defined by the expression  $\frac{\log_{10} I_0}{l}$  where  $I_0$  is the intensity of radiation through the solvent,  $I$  is the intensity of radiation through the solution, and  $l$  is the cell thickness.) The results are shown in Table I. The value of 326  $m\mu$  in the ultraviolet has been chosen because the recent work of Zscheile and Henry (11) and of Morgareidge (7) has shown this to be closer to the absorption maximum of vitamin A than the previously used 328  $m\mu$ .

A series of pure  $\beta$ -carotene samples (obtained from Dr. Salmon, Alabama Agricultural Experiment Station) dissolved in redistilled Skellysolve B, had been set aside in tightly stoppered test tubes, in the dark, at different temperatures, in order to study the kinetics of isomerization to the neo- $\beta$ -carotene reported by Beadle and Zscheile (2). When the analyses of these samples

were made for the per cent of isomerization to neo- $\beta$ -carotene the optical density at 326  $m\mu$  was also determined.

The carotene was extracted from the alfalfa in a Waring Bendor with an alcohol-Skellysolve B mixture. The extract filtered, washed with water, extracted three times with Skellysolve B, concentrated to approximately 60 ml., dried over sodium sulfate, and adsorbed on a column of 2 parts of H Super-Cel and 1 part of magnesia (Micron brand No. 26). The carotene was separated from the xanthophylls and chlorophylls by elution with a 4% acetone-Skellysolve B solution. This is, essentially, the method of Moore and Ely (6), as modified by Wall and Kelley (9). This fraction, no doubt, contains a small percentage of  $\alpha$ -carotene. Since acetone exhibits considerable absorption at 326  $m\mu$ , it was removed from the eluate by washing with water. The purified extract was then dried over anhydrous sodium sulfate before making absorption measurements.

Figure 1 shows that a straight-line relationship exists between the extent of  $\beta$ -carotene isomerization and the calculated optical density ratios; and, therefore, explains the apparently anomalous correction values which have been reported. The values obtained in this study include the range of corrections previously reported. It would appear that the density ratio for pure carotene is about 5.0% in Skellysolve B. Estimating from data given by Zechmeister and Polgár (10), the value is about 5.0% in hexane. The equation of the line in Figure 1 is:

$$\frac{D_{326 \text{ m}\mu}}{D_{436 \text{ m}\mu}} \times 100 = 5.0 + 0.480 \times \% \text{ "neo-}\beta\text{-carotene"}$$

Care in the use of reported correction values is necessary. A shift in absorption maxima in various solvents is well known. The type of instrument on which the calibration is made is of importance. Such factors as slit width and scattered radiation will also undoubtedly influence correction values. The data presented here were taken on the Beckman (3) spectrophotometer, using slit widths of 0.02 mm. at 478  $m\mu$ , 0.04 mm. at 436  $m\mu$ , and 0.34 mm. at 326  $m\mu$ .

## SUMMARY

The isomerization of  $\beta$ -carotene is at least partially responsible for the wide range of correction values reported for vitamin A analysis in the ultraviolet. The correction required at 326  $m\mu$  for  $\beta$ -carotene in Skellysolve B has been calculated on the basis of data taken on the Beckman spectrophotometer and shown to be a linear function of the per cent isomerization.

Table I. Effect of  $\beta$ -Carotene Isomerization on Absorption at 326  $m\mu$

Sample	Optical Density			"Neo- $\beta$ -Carotene", %	$\frac{D_{326}}{D_{436}} \times 100$
	436 $m\mu$	478 $m\mu$	326 $m\mu$		
$\beta$ -Carotene					
Sample 1	0.730	0.781	0.109	18.3	14.9
Sample 2	0.692	0.762	0.075	12.5	10.8
Sample 3	0.611	0.704	0.036	2.2	5.9
Sample 4	0.597	0.690	0.031	1.5	5.2
Sample 5	0.988	1.030	0.156	24.5	15.8
Sample 6	0.900	0.898	0.176	33.8	19.6
Sample 7	0.678	0.779	0.048	3.0	7.1
Sample 8	0.641	0.744	0.040	1.0	6.2
Alfalfa leaf extract	0.805	0.842	0.138	24.0	17.1
Alfalfa leaf extract	0.256	0.286	0.026	9.4	10.1
Alfalfa leaf extract (refluxed 30 hours)	1.293	1.267	0.316	37.5	24.4
Alfalfa leaf extract (refluxed 30 hours)	0.825	0.811	0.187	36.5	22.7



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# Apparatus for Rapid Polarographic Analysis

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VARIETY of different types of cells, each of which has its merits, has been proposed for polarographic analysis (3). Fundamentally, these all fall into two categories; (1) those in which a quiet pool of mercury in direct contact with the solution being analyzed constitutes the second electrode, which were recommended originally by Heyrovský and much used in the earlier work with the dropping electrode, and (2) those in which a saturated calomel, or other nonpolarizable reference electrode, is used as the second electrode. For reasons already discussed (3) the latter type, such as that described by Lingane and Linen (5), are preferable for general use, particularly in research studies when the polarographic behavior of a substance is being investigated for the first time. However, for more or less routine analyses of substances of well-known polarographic char-

acteristics cells of the first type often are more convenient. The cell shown in Figure 1 has proved to be very useful for a variety of analyses.

## SIMPLIFIED POLAROGRAPHIC CELL

In this simple cell the inconvenient classical mercury pool anode has been replaced by a silver-silver chloride anode, which consists of No. 22 silver wire wound as a tight cylinder directly on the dropping electrode capillary, as shown, with its free end spiraled up to the rubber connecting tube where it is held in place by a wrapping of copper wire. The silver wire cylinder is about 2 cm. long, and its lower end extends to within about 3 or 4 mm. from the tip of the dropping electrode. To ensure a reproducible potential, it is advisable to deposit electrolytically a thin coating of silver chloride on the silver electrode before use. The apparent area of the electrode is about 5 sq. cm., which is amply large to prevent appreciable polarization. As a matter of fact, the area of the electrode immersed in the solution may be as small as 1 sq. cm. without significant polarization occurring with currents of the usual magnitude.

This silver-silver chloride electrode may be employed whenever the solution investigated contains chloride ion, and when it does not contain substances which will dissolve silver chloride (metathesis of the silver chloride to a more insoluble salt is permissible). For example, it may be used with any of the common supporting electrolytes containing alkali or alkaline earth halides, hydrochloric acid, acidic, neutral, or basic tartrate solutions containing chloride ion, sodium hydroxide, in solutions of the tetra-alkylammonium halides or hydroxides, etc. The electrode may not be used in ammoniacal solutions, in cyanide solutions, or in general whenever the solution contains substances that form very stable complex ions with silver, because in such cases the silver chloride coating will be dissolved and the polarogram will show a diffusion current of the silver complex. A safe criterion that may be applied in doubtful cases consists of adding a drop or two of 0.1 N silver nitrate to about 10 cc. of the solution to be investigated, and if a precipitate forms (it need not be silver chloride) the silver chloride electrode may be used safely.

The potential of the silver-silver chloride electrode in any particular medium may be determined either by direct measurement against the saturated calomel electrode (for which purpose an H-cell with saturated calomel anode, 5, is convenient), or by comparing the apparent half-wave potential of some substance as determined with the silver-silver chloride anode with the known value referred to the saturated calomel electrode. The potential of the silver-silver chloride electrode is subtracted algebraically from observed half-wave potentials to refer the latter to the standard saturated calomel electrode. In any given chloride-containing medium the potential of the silver-silver chloride electrode is 0.046 volt more negative than that of a calomel electrode in the same solution.

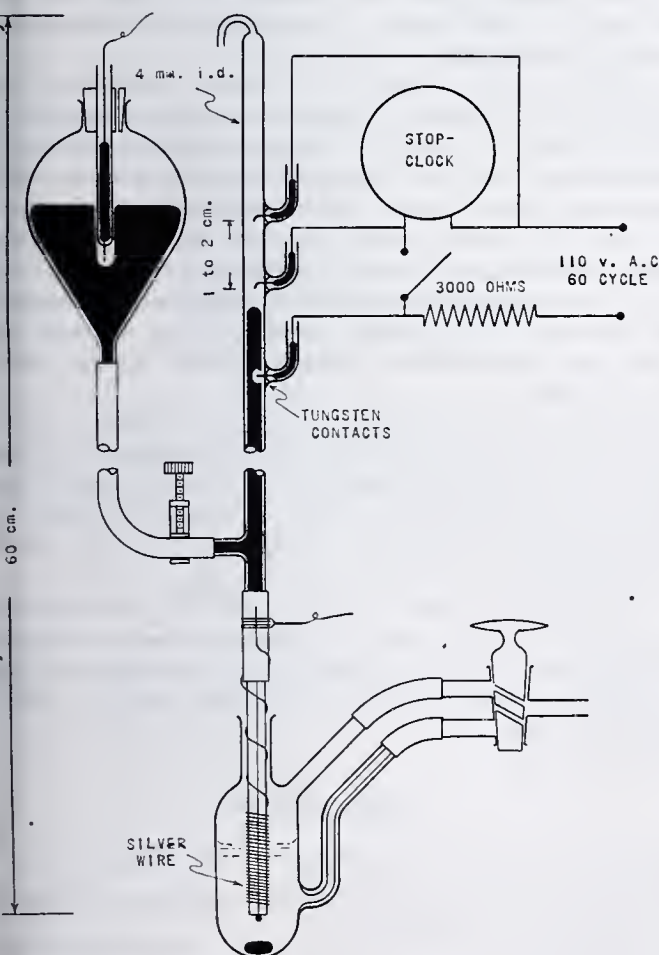


Figure 1. Polarographic Cell



The cell is provided with two gas-inlet tubes, the lower of which is constructed of capillary tubing to facilitate the formation of small gas bubbles and thus provide a large contact surface between the gas and the solution. Nitrogen or hydrogen is first passed very rapidly through the lower inlet tube to free the solution from dissolved oxygen, and then it is passed at a slower rate over the surface of the solution by means of the upper inlet tube during the recording of a polarogram.

This cell is convenient because it requires only a small volume of solution (3 to 7 cc.), but more importantly because it permits very rapid removal of dissolved air from the solution. This latter advantage is demonstrated by the polarograms in Figure 2.

In this experiment, 5 cc. of a 0.465 millimolar solution of cadmium ion in 0.4 *M* sodium tartrate, 0.1 *M* sodium hydrogen tartrate, 0.1 *M* sodium chloride, and 0.005% gelatin, were used. Curve *a* is a polarogram of the solution before removal of dissolved air, and it shows a large wave of oxygen prior to the cadmium wave. Curve *b* was recorded after a very rapid stream of pure nitrogen was passed through the solution for only 1 minute, and it is seen that in this short time about 90% of the oxygen was removed. Curve *c* was recorded after a rapid stream of nitrogen was passed for a total of 3 minutes, and curve *d* was obtained after a slower flow of nitrogen had been maintained for an hour longer. The fact that there is no significant difference between curves *c* and *d* shows that removal of dissolved oxygen was complete after only 3 minutes. The practical advantage of being able to remove oxygen this quickly is obvious.

#### AUTOMATIC DETERMINATION OF RATE OF MERCURY FLOW FROM DROPPING ELECTRODE

The stop-clock circuit sketched in Figure 1 determines automatically the rate of mercury flow, *m*, from the dropping electrode, which datum is required in applications of the Ilkovič equation (3), and especially for the use of diffusion current constants in practical analyses (4). The operational principle of this device is essentially the same as that employed by Feskov (2) and Drake (1) for automatic measurements with gas effusimeters—namely, that the flow of a conducting liquid through a tube past fixed metallic contacts automatically starts and stops an electric stop clock, which thus registers the time required to empty or fill the volume between the contacts.

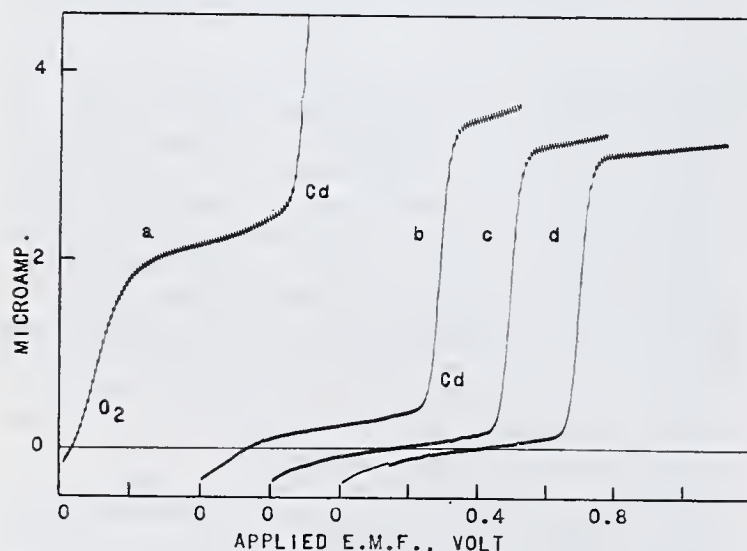


Figure 2. Polarograms

Referring to Figure 1, three tungsten contact points are sealed into the stand tube above the dropping electrode; the two lower contacts complete the circuit through the clock, while the uppermost short-circuits the clock and prevents it from running. The stand tube is constructed from Pyrex tubing 4 mm. inside diameter. The two upper contacts are placed between 1 and 2 cm. apart, and the lower contact is sealed in at any convenient point below the middle contact. To minimize the tendency for the mercury

to adhere to the contacts, they should be cleaned carefully, to a fine point, bent downward as shown, and located as exact as possible in the center of the tube.

A 3000-ohm resistance was placed in series with the clock to minimize sparking at the contacts. This resistance should be as large as operation of the clock will permit, and its magnitude depends on the characteristics of the clock that is used. The author used a precision stop clock manufactured by the E. Thompson Clock Co., Bristol, Conn., but an ordinary inexpensive clock which will start and stop without more than 1-second lag on coast, and which will allow the use of a series resistance of at least 1000 ohms, should be equally satisfactory. The switch shown in Figure 1 is provided to permit ordinary use of the device for other measurements.

A measurement is started by raising the mercury reservoir so the mercury level is a few millimeters above the upper contact. The screw clamp on the rubber tube connecting the mercury reservoir with the stand tube is then closed tightly, the stop clock is set to zero, and the apparatus is left to itself. When the mercury level falls below the upper contact the short-circuit is broken and the clock starts. Then when the mercury level leaves the middle contact the circuit through the clock is opened and it stops. Thus, the clock registers the time of flow of that weight of mercury that is equivalent to the volume between the two upper contacts, and *m* is obtained by dividing this previously determined weight in milligrams by the registered time in seconds. By merely opening the screw clamp to reset the mercury level, the device is ready for another measurement.

The precision with which this device measures *m* is demonstrated by the following successive readings obtained with the same dropping electrode: 505, 506, 510, 507, 509, and 508 seconds. The average deviation of these values from the mean (507.5 seconds) is  $\pm 2$  seconds, or  $\pm 0.4\%$  and the largest discrepancy between any two readings is 5 seconds, or 1%. Since polarographic diffusion currents are measurable with an accuracy of about  $\pm 1\%$  at best under practical analytical conditions, since the diffusion current is a function of the two-thirds power of *m*, it is evident that this instrument is amply accurate for general use. By placing the contacts closer together and/or using a narrower tube the time of measurement could be shortened, but since the measurement is made automatically the time is not important, and it is preferable for it to be at least 500 seconds to ensure satisfactory accuracy.

Calibration of the instrument is accomplished most conveniently by determining the *m*-value of a dropping electrode in the ordinary way (3) from the weight of mercury delivered in a measured time, and then clocking the same electrode with the instrument. In this manner the dropping electrode which yielded the above time measurements was found to have an *m*-value of  $4.056 \pm 0.006$  mg. sec.<sup>-1</sup> at 25° C. Therefore, the weight of mercury corresponding to the volume between the upper contacts of this particular tube was  $2060 \pm 8$  mg., and in an unknown case *m* is obtained by dividing this weight constant by the registered time in seconds.

Since *m* is a linear function of the height of mercury in the stand tube (3), the value determined by this instrument is an average value corresponding to a mercury level exactly midway between the upper contacts. Hence the mercury level is adjusted to this point, with the pinchclamp open, during actual use of the dropping electrode.

The temperature coefficient of *m* is  $+0.31\%$  per degree at room temperature (4), and therefore it should be measured with the dropping electrode immersed in water or solution at the same temperature (preferably 25° C.) at which the electrode will subsequently be used.

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# Fluorocolorimetric Determination of Blended Oils and Oil in Oil-Water Emulsions

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THE past few years the use of fluorescence in both qualitative and quantitative analyses has advanced rapidly. Many photoelectric and fluorescent instruments and techniques have been developed and are now available to the analyst. The application of this principle to analysis has speeded up and made more accurate the determination of many elements and compounds.

The author of this article was called upon to determine the concentration of sulfurized cutting oils blended with light mineral oil used as a cutting coolant and also the content of oil-water emulsions.

The only method available for cutting oils was by means of viscosity, a slow and messy procedure. It was necessary to make up definite concentrations of the blended oils and determine viscosities of these standards, after which the viscosities of the samples themselves were determined; a procedure, while perhaps accurate, was certainly time-consuming.

In the case of the oil-water emulsions, it was found necessary to break the emulsion by the use of large quantities of sulfuric acid and then measure, by volume, the amount of oil in the samples. Both methods were very slow, and the latter was inaccurate.

## DETERMINATIONS BY FLUORESCENCE

In order to improve the procedures and speed them up, use was made of the phenomenon of fluorescence excited by ultraviolet rays. Determinations by this means could be made in a few minutes as the older ones took in hours and were applicable to oils and waxes which exhibited any degree of fluorescence, and waxes which did not fluoresce could be estimated in the same manner by adding suitable oil-soluble fluorescent dyes.

Ultraviolet rays were supplied by a bank of four 2-watt argon tubes, the visible rays being filtered out by a Wratten ultraviolet filter. Strips of blotting paper,  $0.6 \times 3.75$  cm. ( $0.25 \times 1.5$  in.), were impregnated with standard samples of the oils made up of definite concentrations, and these, because of the fluorescence of the oil itself, produced a sharp gradation of colors. It was then an easy matter to match the samples to these standards. The concentrations of the various blends can be related graphically to the viscosity, it is only necessary, when desired, to refer to an appropriate curve to express the fluorescent-colorimetric determination in terms of Saybolt seconds.

To determine the concentrations of oil in the oil-water emulsions, samples of known oil concentrations were prepared and the unknown sample was colorimetrically compared with them. The standards could be used indefinitely, and did not deteriorate over periods of time.

## EXTRACTION METHOD

Many emulsions are subject to contamination that might interfere with the visual colorimetric method and lead to erroneous results. In order to remove the interfering parts of the sample, the following extraction method was developed:

Two milliliters of the sample were placed in a large stoppered test tube and 10 ml. of ether added. This was well shaken, and 25 ml. of a saturated solution of sodium chloride were added to break the emulsion.

A small portion of the ether layer, now containing all the oil in the sample, was placed in stoppered glass tubes 0.47 cm. ( $\frac{3}{16}$  inch) in diameter. It was not necessary to measure the volume of this portion of the sample, as each tube was of equal size and all held the same volume.

Standards treated in exactly the same manner were prepared and colorimetric comparisons made. It was necessary to prepare only one set and seal them well in order to prevent evaporation of the ether, as these could be used indefinitely.

Very close estimation of the total oil content in emulsions could be made by this method, as any error caused by evaporation of the ether was negligible and no measurable trace of

oil remained in the aqueous layer. Determination of oil in oil emulsions could be performed, by visual matching, to  $\pm 0.1\%$  (12.5 parts in 10,000).

In the case of blended oils, all samples when checked colorimetrically with viscosity, showed a difference of only  $\pm 2$  Saybolt seconds. Figure 1 shows the relationship of Saybolt seconds to composition of blended oils in terms of ratios—i.e., the proportions of blending agent to mineral oil. This difference brings a maximum error of only 3% at the lower end of the graph and much less at the higher end. As the usual ratio of blending agent to mineral oil lies about the center, the error is reduced proportionately. The blended oils referred to do not contain water; they are not emulsions.

In the estimation of emulsions by means of the ether-extraction method, smaller samples gave a much sharper color gradation.

This principle was also applied to determine the efficiency of oil removal when metal parts were cleaned and washed by various methods. A trace of oil not visible to the naked eye became apparent when excited by ultraviolet rays.

## ACKNOWLEDGMENT

The author must express his gratitude to Albert W. Bull, without whose assistance and criticism this method could not have been developed.

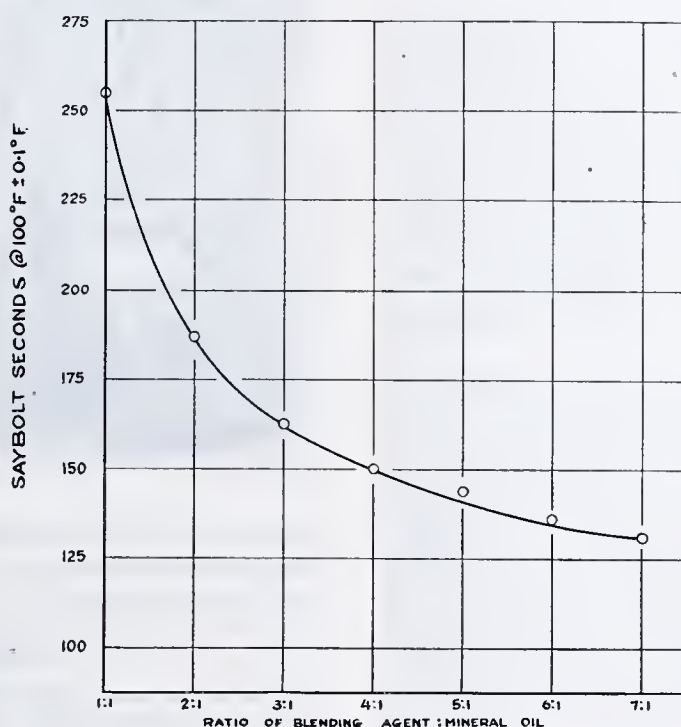


Figure 1. Viscosity of Cutting Oil



# A Versatile Continuous Laboratory Extractor

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**I**N CONNECTION with a project carried out in this laboratory, it was necessary to extract a fairly large quantity of various crude materials with organic solvents, such as ether, acetone, methanol, and benzene. It was desired to make the extractions approximately quantitative. The ordinary size of Soxhlet extractor does not have the capacity required, while large all-glass Soxhlet extractors are expensive. The extractors of Clarke and Kirner (1) and Tanner (3) were considered not rapid enough and subject to loss of solvent vapor, respectively.

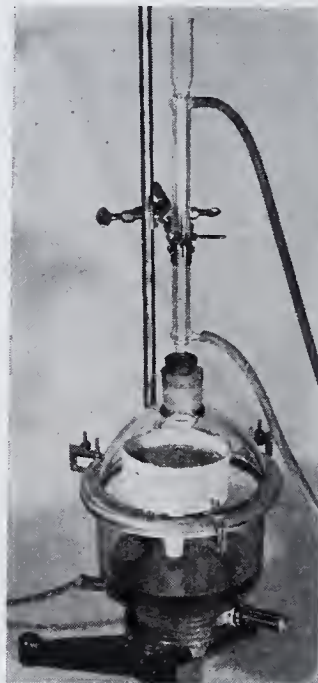
An improved extractor constructed from apparatus found in the laboratory possesses certain unique advantages.

As shown in the photograph, the extractor is made up essentially of a distilling flask, a Büchner funnel, and a water-cooled condenser. The distilling flask, which is a standard item (Pyrex Catalog 3480), consists of two parts: a heavy Pyrex bottom with a wide ground rim, and a Pyrex dome with a wide ground flange and an opening at the top. The bottom of the flask has an inside diameter of approximately 165 mm., is 105 mm. deep, and has a capacity of 2000 ml. The dome is preferably fitted with a standard taper joint at the top, although the one shown is not so constructed. The Büchner funnel has an outside diameter of 106 mm. and is supported in the flask on a small tripod, which is easily made from wire or glass rod. It is necessary to cut off about 3 cm. of the stem of the funnel in order to fit the funnel into the flask.

About 300 to 500 ml. of solvent are put into the bottom of the flask. The funnel is fitted with a sheet of filter paper and 100 to 200 grams of the material to be extracted are added. The two parts of the flask are sealed by a rubber gasket or by a lubricant unaffected by organic solvents (2). A Thiokol gasket previously extracted with the solvent to be used makes a good seal. When a gasket is used, the two sections of the flask are held together by three Hoffman clamps placed around the flange. The flask is heated by a thermostatically controlled electric hot plate which may for safety be covered with an asbestos pad, although no breakage has been encountered with direct heating. The rate of heating is so adjusted that the surface of the material being extracted is at all times covered with a thin layer of solvent. Under these conditions there is a constant flow of solvent through the material, thereby preventing restricted flow through a depression caused by the drip of the condensate.

If the material being extracted is very porous, the use of more than one sheet of dense filter paper will hold up the solvent efficiently to keep the material covered with solvent.

After the extraction is complete, the funnel and tripod are removed, a distilling flask is connected to the flask, the condenser is arranged for distillation to remove the solvent. When all the solvent has been removed, the apparatus is dismantled, and the bottom of the flask is placed in an oven or desiccator in order to dry the extract. The large surface presented by the bottom of the flask facilitates drying, particularly with viscous liquid extracts.



When used in the organic chemical laboratory, the extractor possesses the unique advantage of permitting the following sequence of operations to be carried out in the same apparatus: the crude material is collected by filtration in the Büchner funnel; the funnel is transferred to the extraction flask and the crude material is extracted; and

the extract is freed from solvent and dried. Such continuity prevents loss of material, saves time, and makes possible approximate quantitative work.

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# A Support for Flasks on Steam and Water Baths

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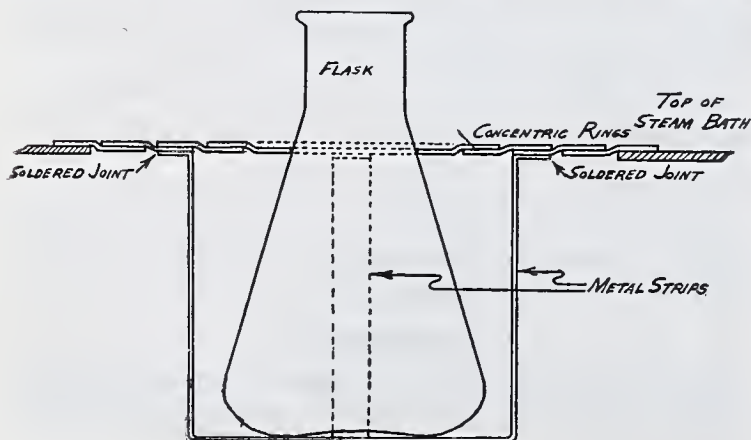
**T**HE refluxing and distillation of solvents in flasks on steam baths are often rather slow, but can be considerably speeded up by lowering the flasks into the bath so that a greater part of the surface is exposed to the steam. The concentric rings which ordinarily form the covers of steam baths allow no more than the

bottom half of round flasks and only the flat bottom of Erlenmeyer flasks to be exposed to the steam, unless the flasks are supported by a buret clamp or some other means.

A simple attachment to the covers is shown in the accompanying illustration. It consists of two strips of sheet metal bent into a U-shape as shown, and soldered to the lower part of the bottom surface of the concentric ring, having an inside diameter larger than that of the flask to be supported. The two U-shaped strips are soldered to the ring at right angles to each other, that they form a rack to support the flask.

The depth of the sides of the U-shaped strips is such that when the flask is in position the next smallest ring or set of rings may be dropped into place over the neck of the flask and will closely surround the narrow upper portion of the flask and also the other rings.

The greater portion of the flask is enclosed by the steam bath and covers and is exposed effectively to the steam, without the necessity of outside supports. The covers serve as a seal around the upper portion of the flask and no other packing is required to stop escape of steam from the system.





# Determination of Cadmium in Biological Material

## Spectrographic, Polarographic, and Colorimetric Methods

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able spectrographic, polarographic, and colorimetric methods the determination of cadmium in biological material are described. Each method cadmium is first separated from the bulk of the wetted matrix by extraction with di- $\beta$ -naphthylthiocarbazone (or dithizone) in chloroform.

As little as 0.4 microgram of cadmium may be determined spectrographically. This method is the most specific and the most rapid when many samples are analyzed routinely. The polarographic method is the most convenient for the analysis of occasional samples and is capable of detecting 1.5 to 500 micrograms or more per 3 ml. of solution. Its specificity is excellent. The colorimetric method is the

TERMINATION of the quantities of cadmium in the tissues, body fluids, and excreta of experimental animals and exposed human subjects necessitates the use of analytical methods of high sensitivity and specificity. The colorimetric method in which ultraviolet intensification is employed lacks sensitivity. A sensitivity of detection of 1/2,500,000 has been claimed for it (8), but Prodan (15), using the method, failed to detect cadmium in 62- to 117-gram samples of blood from cats killed immediately at the termination of feeding experiments with cadmium salts. This was particularly striking in view of the reported high concentrations of the metal in the urine, kidneys, and liver of these animals.

Polarographic, spectrographic, and colorimetric methods employing dithizone have been used to detect small amounts of cadmium in various industrial products and raw materials, but apparently these methods have not been applied to the determination of cadmium in biological materials. Spectrographic detection was found possible with as little as 0.4 microgram of cadmium in the direct current arc (4), while as little as 0.5 to 1 microgram per milliliter of solution can be detected with the spectrograph if instruments are employed with which it is possible to eliminate or reduce galvanometer oscillations (12). Fischer claims for the dithizone method a sensitivity of detection of 0.1 microgram of cadmium in nonferrous alloys, and Sandell has applied Fischer's method to the analysis of igneous rock containing 10<sup>-5</sup>% cadmium. Sensitive methods for the detection of traces of cadmium have also been reported by Mahr (13) and by Dwyer (7), but these methods cannot be used for quantitative determination of the element.

This paper, therefore, concerns itself with the modifications and details necessary to adapt spectrographic, polarographic, and colorimetric methods to the determination of cadmium in biological material in any range of concentration likely to be encountered in such material.

### PROCEDURES

PREPARATION OF SAMPLES AND INITIAL CONCENTRATION OF CADMIUM. The sample, 50 to 100 ml. of urine, 5 to 20 grams of feces, dried feces, blood, or other material, is wet-ashed by a sulfuric acid-nitric acid mixture in an open beaker, Kjeldahl digestion, or closed digestion system such as has been used for arsenic analysis (10). The digest is evaporated to fumes of sulfuric acid, any char occurring meanwhile being destroyed by repeated addition of small portions of nitric and perchloric acids. When ashing is complete, the cooled sample is rinsed into a clean 100-ml. graduated Squibb-type separatory funnel. Fifteen milliliters of ammonium citrate solution (400 grams of citric acid in 600 ml. of water made alkaline to phenol red with con-

most sensitive, but it is laborious and time-consuming, and requires careful attention to details if contamination by zinc is to be avoided. Cadmium is separated from lead and zinc by an extraction with dithizone solution from 5% sodium hydroxide solution. Entrained zinc, lead, and bismuth are then removed by washing the extract with water. Loss of cadmium to the wash water is prevented by adding an excess of dithizone solution to the cadmium dithizonate extract. The cadmium is estimated colorimetrically from a mixed color phase, di- $\beta$ -naphthylthiocarbazone in chloroform being employed for this purpose. Fractions of a microgram of cadmium can be detected and estimated.

concentrated ammonia and diluted to 1000 ml. with distilled water) are added and the volume is made up to 50 ml. with distilled water. After the addition of 2 drops of phenol red indicator, concentrated ammonia is added until the indicator just changes to pink (pH 8.3).

The cadmium, zinc, lead, and other metals are then extracted by adding 5-ml. portions of di- $\beta$ -naphthylthiocarbazone (or dithizone) in chloroform (200 mg. in 1000 ml. of chloroform). Each 5-ml. portion is removed to a second funnel before the next portion is added, and the extraction is continued until the last 5-ml. portion added shows no change in its original color. The combined chloroform extract is shaken with 50 ml. of distilled water, and is then removed to another funnel. The aqueous layer is shaken with 5 ml. of clear chloroform and this too is added to the chloroform extract. The chloroform extract is next shaken with 50 ml. of 0.2 *N* hydrochloric acid and is discarded, while the aqueous layer is washed with pure chloroform in order to remove entrained di- $\beta$ -naphthylthiocarbazone. In case polarographic or spectrographic estimation is to be made, the aqueous layer is then transferred quantitatively to a small beaker and is allowed to evaporate to dryness on the hot plate. For the colorimetric method, the aqueous portion is retained in the funnel and the procedure described below under "Colorimetric Method" is followed.

SPECTROGRAPHIC METHOD. To the dried residue in the beaker, 1 ml. of a salt buffer solution (1% solution of disodium acid phosphate, sodium chloride, or urine salt stock, 3, containing 10 mg. of molybdenum as sodium molybdate per 100 ml.) is added, and 0.2 ml. of the resulting solution is placed in a crater (3  $\times$  10 mm.) drilled in a 2.5-cm. (1-inch) length of 0.78-cm. (<sup>5</sup>/<sub>16</sub>-inch) graphite rod. The rod is dried in an oven and is then used as the lower positive electrode of a direct current arc, the upper electrode consisting of a 3.75-cm. (1.5-inch) length of rod, one end of which is turned to a point in a pencil sharpener. The arc is operated for 2 minutes at 10 amperes from a 110-volt direct current line, and the spectrum is photographed on an Eastman No. 33 plate at setting 4 of the large quartz Littrow spectrograph. Each analytical spectrum is obtained with a rotating 5-step sector (factor 2) set before the slit of the spectrograph. The developed and dried plates are then photometered and partial H and D curves are plotted for the cadmium line at 3261 Å., and for the molybdenum (internal standard) line at 3209 Å. The log exposure separation between the two curves at *T* = 0.50 (*D* = 0.30) is then obtained and the concentration of cadmium is read from a predetermined calibration curve derived from spectrograms of known amounts of cadmium in 1-ml. portions of the salt buffer (4). This technique is satisfactory for the determination of 5 to 200 micrograms of cadmium in 1 ml. of the salt buffer.

For the determination of 2 to 5 micrograms of cadmium per milliliter of buffer solution, corrections for plate background must be made. The technique for this procedure, involving intensity ratios, has been described in an earlier paper (5). In this low range, the cadmium line in the step representing the maximum exposure and the molybdenum line in the third step of each stepped-spectrogram, are employed.







st to dithizone, di- $\beta$ -naphthylthiocarbazone and its metal complexes are insoluble in the alkaline aqueous phase and therefore fewer extractions are required (particularly at higher pH values) to remove the metal complexes completely. This insolubility is disadvantageous, however, in the step of the colorimetric procedure in which the cadmium is separated from the zinc and lead by extraction from the strongly alkaline aqueous phase, since all metals forming complexes with di- $\beta$ -naphthylthiocarbazone are also extracted. For this isolation, therefore, dithizone must be used. Di- $\beta$ -naphthylthiocarbazone is again used in the final colorimetric step in which only pure cadmium is present, since its insolubility eliminates the need for a strict control of pH in obtaining a stable and reproducible zero point. Dithizone partitions more readily between the chloroform and alkaline aqueous phases, and a strict control of pH is required to obtain reproducible results.

The colorimetric method, although the most sensitive (as little as 0.01 microgram of cadmium can be detected, 9), is hindered by a number of difficulties which are eliminated or reduced only by careful attention to detail. The greatest danger is in the fact that it is difficult to separate cadmium completely from the zinc which is entrained with the cadmium dithizonate. Scher (9), recognizing this difficulty, advised washing the cadmium dithizonate with a 2% solution of sodium hydroxide. The authors have found that while this treatment is effective in removing zinc, it also results in a loss of cadmium (1 to 5 micrograms), especially when less than 20 micrograms of cadmium is present. The same loss was found to occur if distilled water was substituted for the alkali, but not if the cadmium dithizonate was stabilized by the presence of excess dithizone. It is for this reason that the dithizone extracts from the 5% alkali are run immediately into a separatory funnel containing 5 ml. of the strong dithizone solution. The wash water is made sufficiently alkaline by removal of sodium hydroxide entrained in the chloroform extract, to extract all the zinc, but only a portion of the excess dithizone. The final washed chloroform phase must contain some excess of dithizone in order to prevent loss of cadmium, and the analyst must use his judgment in determining proper amount of strong dithizone to add in order to make certain that some remains in the chloroform phase. This is especially true when larger amounts of alkali are entrained as a sequence of increasing the number of dithizone extractions in order to make certain that all of the cadmium has been extracted.

Sandell (16) has stated that when nickel is present in the strongly alkaline phase it is slowly extracted by dithizone. It is the authors' opinion, however, that interference on the part of nickel is due chiefly to its oxidative action on weak dithizone solutions. This oxidation may be readily recognized by the fact that instead of the colorless chloroform obtained when cadmium extraction is complete one obtains a yellow shade which is due to oxidized dithizone. In such cases even though the chloroform phase does not become colorless when all the cadmium has been extracted, completeness of extraction may be assumed if the yellow tinge persists in the chloroform phase. That a complete separation of cadmium from zinc and nickel was attained by the authors' method of extraction and washing, was proved by the spectrographic examination of the acid extract of the washed cadmium dithizonate-dithizone phase.

This is illustrated in Figure 2, in which A represents a sample of 10 micrograms of cadmium to which 100 micrograms each of zinc and nickel were added. Extractions with dithizone were made following the addition of 1.25 grams of sodium tartrate, 5 grams of sodium hydroxide, and adjustment of the volume to 100 ml. B represents a comparison polarogram obtained with the evaporated residue of a hydrochloric acid solution to which 10 micrograms of cadmium and 2 micrograms each of nickel and zinc were added. Separation of the nickel and zinc diffusion was accomplished by electrolyzing the residues following their solution in 3 ml. of a solution containing 0.1 *N* ammonium tartrate and 0.025 *N* potassium thiocyanate.

Lead and bismuth are the only other metals which may be extracted with cadmium and zinc and carried along to the step in which the cadmium is isolated. Like zinc, however, lead and bismuth are not extracted by dithizone from strongly alkaline solutions and therefore they are removed along with the zinc in the extraction and washing step. Cobalt, nickel, copper, mercury, and silver originally present in the sample are extracted initially, but all except possibly traces of copper and nickel remain behind in the chloroform phase when the latter is shaken with the 0.2 *N* hydrochloric acid in preparing the extract for separation of the cadmium from zinc and lead. The small amounts of copper and nickel that may be carried to the cadmium-isolation step are not extracted by dithizone from the 5% sodium hydroxide solution.

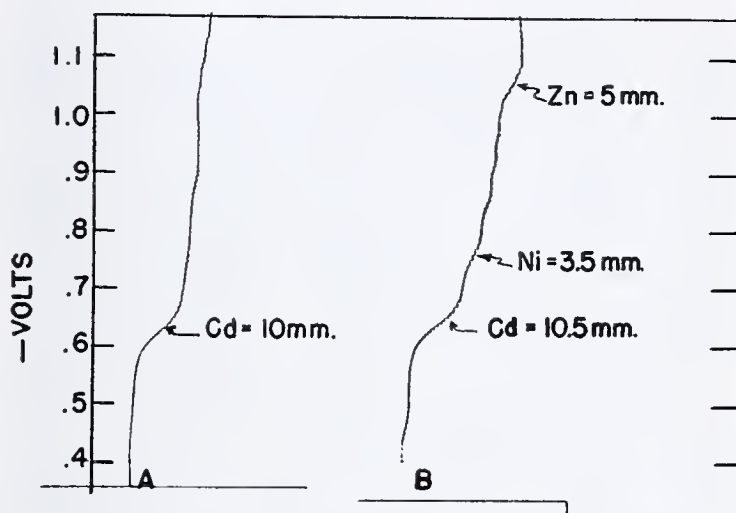


Figure 2. Polarograms Demonstrating Removal of Interfering Zinc and Nickel in Colorimetric Determination of Cadmium

In developing the colorimetric method it was noted that when the dithizone solution made from Eastman dithizone was used in the cadmium-separation step, it imparted a pink color to the chloroform even in the absence of cadmium. This was found to be due to the presence of semicarbazone which, however, was easily removed by purifying the dithizone according to the procedure described by Cowling and Miller (6). It was also observed, in confirmation of Sandell's finding (16), that weak cadmium dithizonate solution (as well as weak dithizone solution) was so unstable that the colors obtained could not be used directly for photometric purposes. This fact forced the authors to lengthen the colorimetric procedure considerably by introducing the mixed-color photometric step, for only in the presence of excess di- $\beta$ -naphthylthiocarbazone (or dithizone) is sufficient stability obtained to permit the reliable estimation of small amounts of cadmium dithizonate by photometric means.

If reliable results are to be obtained with the colorimetric method, great care must be taken to ensure cleanliness of the extraction apparatus, pipets, and other containers used in making the final dilution for photometric reading. If this is not done, zinc present as surface contamination will also give a color which is read as cadmium. If the glassware is properly cleaned by repeated rinsing with dilute nitric acid and distilled water, it is possible to estimate quantities of cadmium in the 0- to 5-microgram range with a sensitivity of  $\pm 0.1$  microgram, and in the 0- to 50-microgram range with a sensitivity of  $\pm 1$  microgram.

The spectrographic and polarographic methods are satisfactory from the standpoint of specificity, sensitivity, and reproducibility (see Table II). Of the two, the spectrographic method is somewhat the more sensitive, and no difficulty is encountered in detecting even 1 microgram of cadmium when the residue of the 0.2 *N* hydrochloric acid extraction is taken up in 0.5 ml. of the buffer salt solution. Results that are accurate within  $\pm 1$  microgram in the range of 0 to 10 microgram per milliliter buffer



**Table II. Comparison of Results Obtained with Spectrographic, Polarographic, and Colorimetric Methods**

(Recoveries of cadmium added to 100-ml. portions of urine)

Cadmium Added Micrograms	Cadmium Recovered		
	Spectrographic method	Polarographic method Micrograms	Colorimetric method
0	1.0	2	1.2
1	2.5	...	2.2
2	3.0	3.5	3.2
5	7	6.5	6
10	11	12	11 <sup>a</sup>
50	48	50	48 <sup>a</sup>

<sup>a</sup> Measured on 0-50  $\gamma$  range.

solution, and within 5% when more cadmium is present, can be obtained by spectrographic means. In the case of the polarograph, it is sometimes difficult to measure accurately the diffusion waves obtained when 1 to 3 micrograms of cadmium are present in 3 ml. of solution, although there is no difficulty in identifying the diffusion wave. Another difficulty occurs at high recorder sensitivity when traces of cadmium are to be determined in the presence of large quantities of lead. At the highest galvanometer sensitivity, it is possible to determine 10 micrograms of cadmium in the presence of a concentration of lead ten times as great. When lead is present in a much greater quantity, the galvanometer sensitivity is too great to permit both waves to appear on the chart. In such cases, the lead may be removed by extraction from the strongly alkaline tartrate solution, as described under "Colorimetric Method". The cadmium is then shaken into 0.2 *N* hydrochloric acid and the polarographic estimation is repeated. Large amounts of bismuth oxychloride also interfere polarographically, but this interference may be eliminated by filtration and re-evaporation of the filtrate to dryness. The interference by bismuth oxychloride may also be eliminated by a re-extraction of the cadmium with dithizone as mentioned above for lead. Other metals such as nickel, cobalt, and zinc give diffusion waves above that of cadmium and consequently do not interfere.

It will be noted from Table II, in which the recoveries by three methods are compared, that cadmium was detected in samples to which no cadmium had been added. From some of the data in Table I, it seemed likely that the considerable quantity of cadmium present in the blank was due to its presence in the reagents. This was proved by analysis of large volumes of sulfuric and nitric acids, for it was found that two thirds of the blank was due to the 10 ml. of sulfuric acid, and the rest to approximately 50 ml. of nitric acid used in each digestion.

In the matter of the choice of one of these methods for routine use, the authors have found that the polarographic method is the most convenient, particularly when only occasional samples are to be run. The spectrographic method, in their opinion, offers the most rapid and economical means of analysis, if large numbers of samples are to be handled daily. The colorimetric method, while the most sensitive, is the most laborious and time-consuming, but provides a reliable means for determination of cadmium when polarographic or spectrographic equipment is not available.

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## Spectrophotometric Determination of Leuco Crystal Violet after Oxidation with Benzoyl Peroxide

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A method is reported for determining leuco crystal violet by oxidizing with benzoyl peroxide and measuring, by means of the spectrophotometer, the intensity of the color formed. The method has a precision represented by a standard deviation for a single value of  $\pm 0.25\%$  of the total leuco crystal violet. The effect of impurities upon the accuracy is discussed.

ONE method described in the literature for the production of crystal violet involves the initial preparation of leuco crystal violet, *p,p',p''*-methenyltris-(*N,N*-dimethylaniline), followed by oxidation to the finished product. The proper control of the oxidation necessitates a knowledge of the content of the leuco form. As far as the authors know, no method of determining this compound has been published; previous to the development of the method reported here leuco crystal violet had been determined merely by ascertaining the amount of material insoluble in a solution which was about neutral after the acid-insoluble constituents had been removed. Obviously, this method is not

specific for the leuco crystal violet, so that it became desirable to find a more satisfactory method. Since the colorless leuco crystal violet is converted to the colored form by oxidation, the possibility of utilizing this behavior as the basis of a colorimetric method was considered.

After a consideration of oxidants, benzoyl peroxide was chosen. A study was then made of the optimum conditions of temperature, time, and concentration for color formation with this oxidant. The detailed method of analysis is given below, followed by a discussion of the experiments which determined the conditions of analysis.

#### METHOD OF ANALYSIS

**APPARATUS.** Most of the color measurements were made using a modified automatically recording Hardy spectrophotometer (2, 3, 4). Calibrated cells, approximately 1 cm. in length, were used. This spectrophotometer plots the spectral curve as  $\log I/I_0$  against a logarithmic function of the wave length, referred to as the octaval and measured in constant resolution units (c.r.u.)



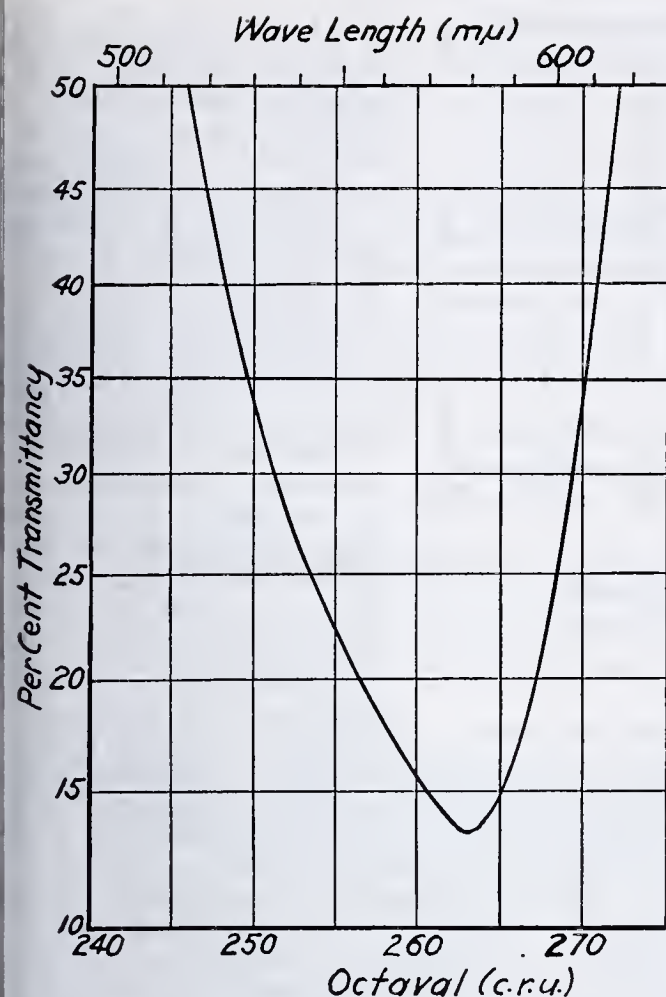


Figure 1. Standard Curve from Oxidation of Recrystallized Leuco Crystal Violet  
 Octaval (c.r.u.) =  $332 \log \lambda \text{ (m}\mu\text{)} - 655$

is latter function can be converted to the more familiar wave-length scale by the equation:

$$\text{Octaval (c.r.u.)} = 332 \log \lambda \text{ (m}\mu\text{)} - 655$$

In order to facilitate the interpretation of these curves, a wave-length scale in terms of millimicrons is shown at the top of Figures 1 and 3.

It should be possible to make the color measurements for this determination with considerable precision with any one of several filter photometers now available commercially. With this type of instrument, a filter transmitting in the range 525 to 580 mμ should be used. The instrument must be calibrated, using a leuco sample of known purity.

**REAGENTS.** Glacial acetic acid, which must be as free as possible from turbidity and colored impurities.

**Benzoyl peroxide solution,** 0.250 gram of c.p. benzoyl peroxide, dissolved and diluted to 100 ml. in glacial acetic acid in a volumetric flask. The solution may be kept for a few days in the dark without obvious deterioration, but must be discarded when it develops a yellow color.

**Caution.** Benzoyl peroxide must not be ground nor exposed to heat because it is said to explode under those conditions.

**PREPARATION OF SAMPLE AND DEVELOPMENT OF COLOR.** The amount of the sample taken for analysis depends upon the leuco crystal violet content. A sample of a weight to contain 0.15 to 0.3 gram of real leuco crystal violet is dissolved and diluted to 10 ml. in a volumetric flask with glacial acetic acid. A 10-ml. aliquot is again diluted to 100 ml. Ten milliliters of this dilution, representing 1.5 to 2.3 mg. of real leuco crystal violet, are transferred to a test tube (approximately 18 × 150 mm.), and 5 ml. of benzoyl peroxide solution are added. The tube is rotated to mix its contents, immersed in a briskly boiling water bath for exactly 4.5 minutes to develop the color, then transferred immediately to an ice bath and shaken for about one minute to cool to about room temperature as rapidly as possible (to prevent overoxidation). Too long cooling is avoided in order to prevent the acetic acid from freezing.

**MEASUREMENT OF COLOR INTENSITY AND CALCULATIONS.** The colored solution which is formed is too dark for direct measurement. Five milliliters are diluted, immediately after diluting, to 100 ml. in a volumetric flask with glacial acetic acid.

The color intensity of this solution is measured within a period of not more than one hour. The transmittancy is measured at 263 c.r.u. (580 mμ) and the amount of leuco is calculated from the following equation:

$$\text{Leuco content (\%)} = \frac{-22.87 \log_{10} T}{\text{length of cell (cm.)} \times \text{wt. of sample (grams)}}$$

In this equation  $T$  is the transmittancy expressed as a decimal. The sample must be diluted as directed in this paper for this equation to hold. Figure 1, the curve upon which this equation is based, was obtained with a 0.2000-gram sample of purified leuco crystal violet after indicated dilutions.

#### COLOR REACTION

**CHOICE OF OXIDANT.** In choosing the proper oxidant, at least three criteria had to be kept in mind. (1) For manipulative convenience, as well, possibly, as for speed of oxidation and ease of controlling the course of the oxidation, it seemed desirable not to use insoluble oxidants such as lead peroxide, which is a type of oxidant actually used for this reaction. (2) To avoid interference with the colorimetric determination, colored oxidants such as permanganate or dichromate should be avoided. (3) The oxidant should not be too vigorous, since there was danger of overoxidation leading to destruction of some of the colored product. Hydrogen peroxide was first suggested, but it was anticipated that this reagent might be too vigorous and lead to destruction of color. Ellinger and Landsberger (1) found during a study of the role of catalysis in biochemical oxidation that crystal violet could be decolorized by hydrogen peroxide in the presence of a number of catalysts. Experiment confirmed this suspicion; it was impossible to get uniform color development with hydrogen peroxide in various concentrations and with various degrees of heating. In fact, the color produced always faded rapidly and often was completely destroyed to yield a colorless solution.

Benzoyl peroxide was then studied. This reagent seems to be a less vigorous oxidant than hydrogen peroxide; it is colorless; and it is soluble in glacial acetic acid, in which both leuco crystal violet and crystal violet itself are also soluble. Furthermore, even under extreme conditions of time and temperature, benzoyl peroxide did not cause sufficient overoxidation to destroy the color completely; at most, a brownish-red coloration was produced by some breakdown of the desired blue color.

**OPTIMUM CONDITIONS FOR OXIDATION.** A study of optimum conditions for using this oxidant showed that by proper adjustment of the concentrations and relative proportions of reductant and oxidant, the time of reaction, and the temperature, a well-controlled oxidation to the violet color could be achieved.

A satisfactory heating time is one which causes a maximum development of color with the least possible decomposition of the oxidized form. This, of course, varies with the temperature. All work was done in a boiling water bath. In attempting to establish the optimum time some determinations were made with heating times varying from 3 to 11 minutes, and all other conditions unchanged. The intensity (absorption peak) of the purple color increased to a maximum, then decreased with longer heating periods. This effect is shown in curves 1, 2, and 3 of Figure 2, curve 1 illustrating the oxidation of purified leuco, and curves 2 and 3 illustrating the oxidation of two commercial samples. Curves representing samples heated for different lengths of time showed another and somewhat different effect in the blue region of the spectrum (Figure 3). The intensity of absorption in this region increased with the time of heating even after the intensity at the absorption peak had actually passed its maximum. This effect is probably due to some decomposition of the oxidized form.

The concentration of benzoyl peroxide and sample size are closely related, since their relative proportions affect the rate of oxidation. Curve 1 of Figure 2 shows the analysis of purified leuco using 12.5 mg. of benzoyl peroxide (as described in the method), curve 4 half that amount, and curve 5 twice that amount. The 12.5 mg. of benzoyl peroxide were selected as the



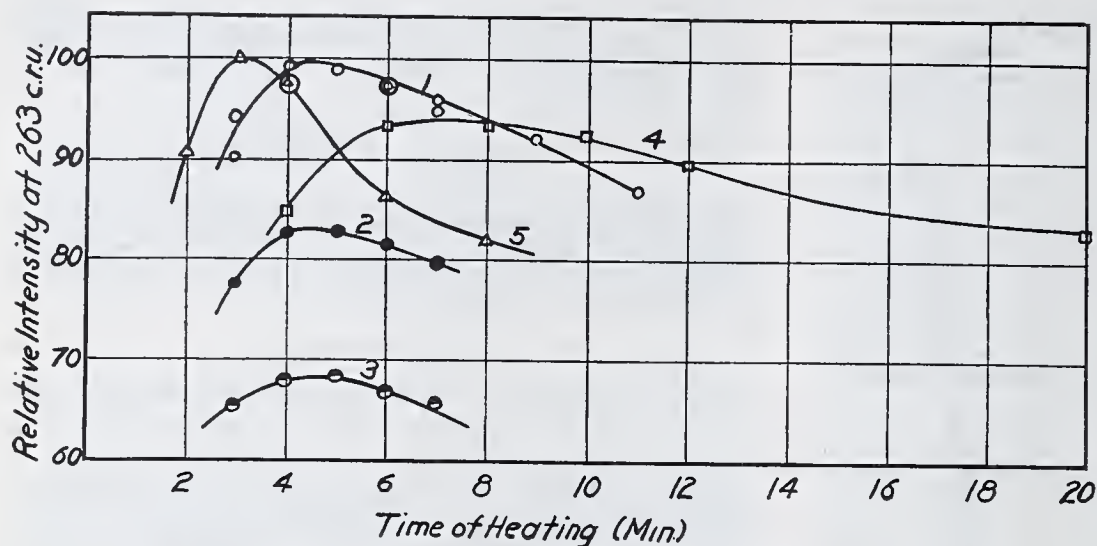


Figure 2. Effect of Time of Heating and Concentration of Benzoyl Peroxide

- 1, 4, 5. Recrystallized samples  
 2, 3. Commercial samples  
 1, 2, 3. 12.5 mg. of benzoyl peroxide per 15 ml.  
 4. 6.25 mg. of benzoyl peroxide per 15 ml.  
 5. 25.0 mg. of benzoyl peroxide per 15 ml.

optimum concentration (for 2 mg. of leuco) because the lower concentration failed to give sufficient color development while the higher concentration, although giving a similar maximum color, required too close an adjustment of the heating period with the possibility of missing the optimum time.

Cutting down the sample size has an effect similar to increasing the benzoyl peroxide concentration. The sample size must be adjusted to contain about 2 mg. of real leuco in order to maintain approximately the same peroxide-leuco ratio used and found to be optimum for the standard. Various weights of a commercial sample which had less than 50% of leuco was analyzed. The values found varied with the sample size as follows:

Sample Size, mg.	Leuco Found in Sample, %
2	39
3	42
4	43

**CHARACTERISTICS AND STABILITY OF COLOR.** The color obtained conforms to Beer's law over the range 50 to 100% in strength at the maximum of the absorption band. Below 50% strength, there is a small negative deviation, which at 25% strength amounts to about 4% of the leuco present. This deviation would not affect an actual analysis, however, since the size of the sample is adjusted so that about 2 mg. of the leuco crystal violet are oxidized.

On aging, the solution gradually becomes weaker at a rate of about 0.5% strength per hour, so that the spectral curve should be determined within one hour after the preparation of the solution.

The color obtained is not affected by irradiation. Exposure of the solution for 3 minutes at a distance of 1 inch from a 100-watt electric light bulb does not affect the curve in any way.

The temperature at which the color is measured does not affect the strength obtained to an extent greater than that which may be accounted for by the thermal expansion of the solvent. No effect was obtained for the range in temperature (25° to 31° C.) in which the color measurements were made.

#### PRECISION AND ACCURACY

From twelve separate complete determinations including all the steps of the method (six determinations on each of two samples), a value for the standard deviation of a single value from the mean was calculated as  $\pm 0.25$  part per 100 of leuco crystal violet. For one sample six values had a range from 84.5 to 85.2% with an arithmetic mean of 84.9%; for the other

sample the six values had a range from 99.8 to 100.5% with an arithmetic mean of 100.0%. The precision is as good as can be expected from the maximum possible sensitivity in reading the curves.

The accuracy of the method is dependent both upon the purity of the leuco crystal violet used as a standard and upon the types of impurities present. The standard used in this work had been recrystallized from ethanol to a constant melting point of 176.6–177.6° (corrected). It was checked further by analyzing it by the method given, together with some of the previous crystallization and some of the subsequent crystallization. The standard had a value of 100.0% a previous crystallization was 98.5%, and the subsequent one was 99.5%. From this it was concluded that the standard had reached a maximum purity.

The second factor which would affect the accuracy would be the presence of impurities which might themselves, or as oxidation products, have an absorption at 263 c.r.u. (580  $\mu$ ). The type of impurities would probably depend upon the particular process used for making the leuco and upon the impurities in the raw materials, so that their effects would have to be studied separately for any given type of sample. In the samples with which the authors worked there seemed to be two general classes of impurities—one which absorbed in the region of 210 to 240 c.r.u. (400 to 450  $\mu$ ), which would not interfere with the method, and another class which absorbed in the same spectral region as the oxidized leuco crystal violet, the peak of the absorption band being about 265 c.r.u. (588  $\mu$ ) against 263 c.r.u. (580  $\mu$ ) for the pure leuco crystal violet. This material was not isolated

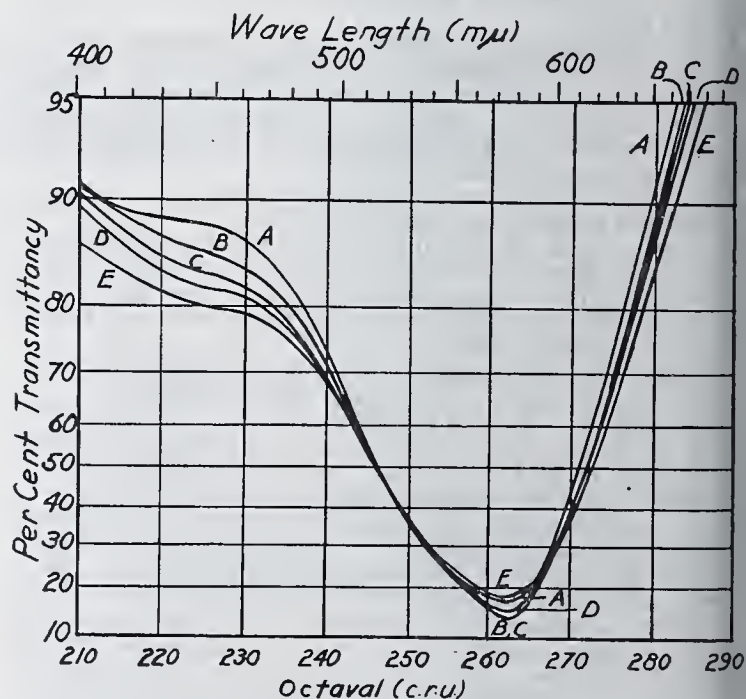


Figure 3. Variation of Transmission Curves with Variation in Heating Time

$$\text{Octaval (c.r.u.)} = 332 \log \lambda (\mu) = 655$$

- A. 3 minutes  
 B. 4 minutes  
 C. 5 minutes  
 D. 7 minutes  
 E. 11 minutes



pure state, so that actually the two peaks may be separated somewhat more than this. In order to correct the results for the presence of this impurity, it would have to be isolated in the pure form and treated in the same manner as the sample. Using the curve for this material, a two-component analysis could be set up the usual way.

The authors did not carry the investigation to the point of isolating and studying the interfering impurities, because the purpose for which the method was needed did not necessitate doing that work. Even without that information, the method marked a considerable advance over that previously used. The values found by the new method were always lower than by the old one.

Crystal violet itself is included in the value reported for leuco

crystal violet, but its presence can be corrected for after determining crystal violet separately without oxidation.

#### ACKNOWLEDGMENTS

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## Microdetermination of Arsenic in Biological Materials

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Pentavalent arsenic may be distilled from a sulfuric acid solution without reduction by the addition of potassium bromide. The distillate contains all the arsenic in pentavalent form suitable for the immediate development of color with ammonium molybdate and hydrazine sulfate. This greatly simplifies the determination of arsenic by colorimetric means. The procedure is given for the determination of arsenic in biological material in the range of 1 to 10 micrograms. Preliminary ashing is done in the usual manner with sulfuric and nitric acids. A special still is used for the distillation to give a small volume of distillate in which the molybdenum color is developed by adding the color reagents directly to the distillate. Antimony does not interfere. Large amounts of phosphorus may interfere, but can be removed by special treatment.

**METHODS** for the determination of arsenic have depended on separation of the arsenic from other metals, either by the evolution of arsine or by the distillation of the arsenic as the chloride. The most satisfactory colorimetric determinations of small amounts of arsenic have depended on the formation of molybdenum blue, a procedure which has been used by a large number of workers (1-5). For this colorimetric procedure the arsenic must be present in pentavalent form, and it is necessary to treat the separated arsenic with some oxidizing agent. Nitric acid has been most commonly used, but this has had several disadvantages. Since the nitrate ion interferes with the color reaction, all traces of the nitric acid must be removed by heating; this must be carefully controlled to prevent losses of arsenic. In addition, considerable time is lost in this step when large numbers of arsenic determinations are done. The method described here is based on the finding that pentavalent arsenic may be distilled as such to give a distillate which contains the arsenic in pentavalent form, without the use of oxidizing agents. Under these conditions the molybdenum blue color may be developed directly on the distillate without further treatment. This modification has given more reliable and consistent results than could be obtained by the method previously described (Chaney and Magnuson, 1), and is more rapid than other published methods. Twenty to thirty determinations may be done each day with no difficulty. The method is most useful in the range of 1 to 100 micrograms of arsenic. While it has been used for the determination of arsenic in biological materials, it could be adapted to other uses.

#### CHEMICALS

All chemicals should be of the best reagent grade. The reagent grade of sulfuric acid (Merck) has been found more satisfactory

than some lots of special arsenic-free grades. Sulfuric acid, concentrated. Nitric acid, concentrated. Perchloric acid, 60% Potassium bromide. Ammonium molybdate. Hydrazine sulfate.

#### REAGENTS

In working with small amounts of arsenic the authors have made up reagents fresh daily to avoid contamination and possible decomposition. They have not determined the keeping qualities of these reagents.

Potassium bromide, 30% solution in distilled water.

Molybdate color reagent. Add 10 cc. of concentrated sulfuric acid to 40 cc. of distilled water, cool, add 1.0 gram of ammonium molybdate, and dilute to 100 cc.

Hydrazine sulfate, 0.05% solution in distilled water.

Standard pentavalent arsenic solution. Dissolve 1.5 grams of arsenic pentoxide in 100 cc. of *N* sodium hydroxide, add 600 cc. of distilled water, neutralize with 100 cc. of *N* hydrochloric acid, and dilute to 1000 cc. Place three 25-cc. aliquots in glass-stoppered flasks and add 25 cc. of concentrated hydrochloric acid and 50 cc. of 10% potassium iodide to each. Make simultaneous blank determinations in triplicate. Allow the flasks to stand in the dark for 2 hours, then titrate the free iodine with 0.1 *N* sodium thiosulfate. One cubic centimeter of 0.1 *N* sodium thiosulfate is equivalent to 3.75 mg. of arsenic.

Make appropriate dilutions from the stock solution to give 10 and 50 micrograms of arsenic per cc. This pentavalent arsenic solution remains unchanged for months.

#### APPARATUS

Round-bottomed two-necked, distilling flasks of 250-cc. capacity, with 24/40  $\text{\textcircled{F}}$  center joint and 19/38  $\text{\textcircled{F}}$  side joint.

Dropping funnel, 19/38  $\text{\textcircled{F}}$  joint to fit 250-cc. distilling flask.

Graham reflux condenser, coil-type, 200 mm. in length with 24/40  $\text{\textcircled{F}}$  joint at either end.

Special still, now available commercially from the Scientific Glass Apparatus Co., Catalog No. M-1586 (Figure 1).

Heater, 350-watt Cenco hot cone. Hot plate. Photoelectric colorimeter. Test tubes graduated at 25 or 35 cc. Erlenmeyer flasks.

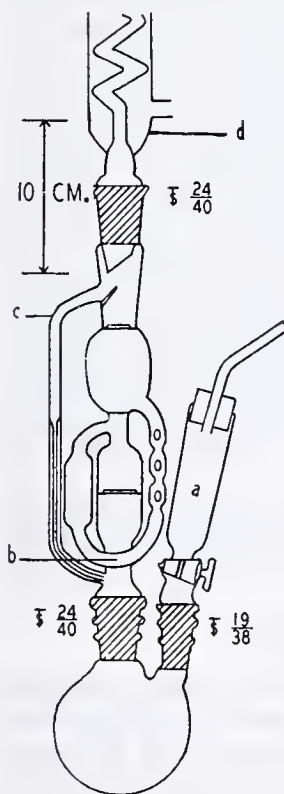


Figure 1. Diagram of Distilling Apparatus



Table I. Recoveries from Reagents

Arsenic Added Micrograms	Arsenic Found Micrograms	Maximum Error %
0	0, 0, 0, 0.1, 0.1	..
1	1.1, 1.1	10
2	2.1, 2.2, 2.0, 2.1	10
3	3.2, 3.2, 2.9, 2.8	7
4	3.9, 3.9, 4.0	3
5	5.0, 4.9, 5.0, 5.0	2
6	6.1, 6.2	3
8	8.1	1
10	9.9, 9.9	1
20	19.9, 19.6	2
30	29.6, 29.6	1
40	39.0, 38.4	4
50	49.3, 49.8	1

## PROCEDURE

The sample is digested in Erlenmeyer flasks, using 5 cc. of sulfuric acid and excess nitric acid as recommended by Morris and Calvery (4). Care should be taken to maintain an excess of nitric acid until all organic matter is destroyed. A few drops of perchloric acid will speed the final stages of the digestion. When the digest is colorless and has cooled, it is transferred to a two-necked distilling flask using two or three 5-cc. quantities of water as a wash. The solution is then heated on the hot cone heater until strong fumes of sulfuric acid appear, in order to remove traces of nitric acid.

**DISTILLATION.** Five cubic centimeters of water are added to the cooled sulfuric acid digest, and 2 cc. of 30% potassium bromide are put in the dropping funnel (*a*, Figure 1), which is then connected to the outer neck of the distilling flask. The distilling head is connected to the center neck and the flask placed on the already hot Hot Cone heater. The flask should rest on the largest removable ring of the heater. When boiling has started and steam has begun to condense in the trap, *b*, 3 cc. of distilled water are added through the top of the still, and the condenser is put in place so that the condensed vapor will drip down the capillary tube, *c*. The potassium bromide is now blown in through the dropping funnel followed by 2 cc. of water as a wash. The distillation is continued for 4 minutes from the time the potassium bromide is blown in; then the still is disconnected from the flask and condenser and the distillate poured out through the top of the still into the test tube in which the color is to be developed. The trap is rinsed two or three times with 2 cc. of water and these rinses are added to the tube. The still itself is rinsed thoroughly with distilled water, and is ready for use again. No further cleaning is necessary.

**COLOR DEVELOPMENT.** The amount of solution in the color tubes following the rinses will be from 15 to 20 cc. To this are then added 2 cc. of the molybdate solution followed by 2 cc. of the hydrazine sulfate. Thorough mixing is essential. These solutions should be added directly to the distillate and not allowed to run down the sides of the tube. The tube is now heated for 10 minutes in a water bath at 90° to 100° C., cooled in cold water, brought to a 25- or 35-cc. volume, and read in a photoelectric colorimeter. The volume of the final dilution will depend upon the type of photoelectric colorimeter used. For the present work the authors have been using a Klett-Summerson instrument employing a cell measuring 2 × 4 × 8 cm. The 4-cm. length is used for values up to 50 micrograms of arsenic and the 2-cm. length for higher values. For this cell the authors have used a final volume of 35 cc. The light filter to be used depends to some degree upon the type of colorimeter. With the Klett a 690-millicon filter has been employed. Where other types of photocells are available, filters of longer wave lengths may be employed. According to Sultzberger (6), the maximum absorption of the compound is at 840 millimicrons.

In setting up undistilled standards of pentavalent arsenic for color development, 3 cc. of normal hydrochloric acid should be added to allow for the amount of acid distilled over in the unknowns.

## TESTS OF THE METHOD

Shown in Table I are recoveries of arsenic added to reagents. The arsenic was added in pentavalent form to sulfuric acid and then distilled. The reference curve was made by developing the color directly on the pentavalent arsenic solutions. In setting up these standard curves 3 cc. of *N* hydrochloric acid were added to the solution in the color tube before the molybdate

was added. With 1 to 3 micrograms of arsenic the recoveries were within 10% of the true values, and above this level recoveries were within 5%. Blank values which range from 0 to 0.2 microgram were subtracted from the recoveries in each instance.

In Table II are shown recoveries of arsenic from whole blood. Known amounts of arsenic were added to 5 cc. of whole blood, digested, and distilled. Blank values were subtracted from the recoveries. Recoveries at the 1-microgram level were within 10%, and above this level were within 5%.

## DISCUSSION

In the course of developing the method, the interesting finding has been made that, contrary to general belief, pentavalent arsenic can be distilled from a mixture of sulfuric acid and potassium bromide and that the distillate contains the arsenic in pentavalent form. This has been shown in two different ways. First, the arsenomolybdate color does not develop unless the arsenic is present in pentavalent form. In the distillates so obtained by the method here described, the color develops without any preliminary oxidation of the arsenic.

Table II. Recoveries from Whole Blood

Arsenic Added Micrograms	Arsenic Found Micrograms	Maximum Error %
0	0.3, 0.3	..
1	1.1, 1.1	10
5	5.0, 5.0, 5.0, 5.1, 5.1, 5.0	2
10	10.1, 9.5, 9.7, 9.5	5
20	19.7, 19.3, 20.0, 19.8	4
40	40.0	0
50	50.2, 50.2, 49.8, 49.8	1
100	100, 102	2

In the second place, arsenic in the distillate does not titrate as trivalent arsenic with iodine, but does titrate as pentavalent arsenic with iodide. Distillates containing 1- to 5-mg. quantities of arsenic were obtained by pooling a number of distillates from the microstill. A number of distillations were also done using a round-bottomed flask connected to a Fresenius flask by a glass tube. Water was used in the Fresenius flask to catch the distillate. Using either method of distillation it was found that pentavalent arsenic were added to the sulfuric acid before distilling, no trivalent arsenic could be recovered in the distillate and 95 to 100% could be titrated as pentavalent arsenic.

When the arsenic was added to the sulfuric acid in trivalent form before distillation, 23 to 30% could be titrated in the distillate as trivalent arsenic and the remainder as pentavalent arsenic.

**TITRATION OF TRIVALENT ARSENIC (6).** The distillate was titrated with 0.01 *N* iodine in the presence of excess sodium bicarbonate. A blank was run simultaneously.

**TITRATION OF PENTAVALENT ARSENIC (6).** Concentrated hydrochloric acid was added to the solution to give a final concentration of approximately 6 *N*, and 5 cc. of 10% potassium iodide were then added. At the end of 2 hours the free iodine was titrated with 0.01 *N* sodium thiosulfate. Blanks were run at the same time and subtracted from the final readings.

The authors have not identified the form in which the arsenic is distilled in the present method. Since the arsenic is present in the digest and in the distillate in pentavalent form, it is probably distilled in the form of an unstable arsenic pentabromide which decomposes on passing through the water in the trap.

The authors have used various types of simple distillation apparatus for determining 50-microgram quantities of arsenic but recoveries have in no case been comparable to those obtained with the microstill. For large quantities of arsenic the microstill was not necessary.



**THE REAGENT BLANK.** Certain lots of sulfuric acid have even difficulty with a reagent blank which is apparently not due to arsenic and is of significance only on determinations below micrograms. With such lots the blank is irregular, and recoveries below 3 micrograms are correspondingly erratic. However, recoveries above this level are perfectly regular, and give no indication of the presence of the blank. Furthermore, to such distilled blanks one adds pentavalent arsenic in excess of 3 micrograms, color develops only in an amount proportional to the added arsenic, and the interfering blank is not observed. The authors have been unable to identify this interfering substance, which is found only in certain lots of sulfuric acid. The most satisfactory sulfuric acid has been the regular reagent grade rather than some of the "arsenic-free" grades.

A "pseudo blank" may also appear if the molybdate solution is added down the sides of the test tube rather than directly to the distillate; the molybdate may decompose on the sides of the test tube and result in a blue color.

**INTERFERING SUBSTANCES.** During the distillation process complete separation from phosphorus is accomplished except for a mechanical carry-over of approximately 1 part in 100,000. Thus, if one adds 0.1 gram of phosphate to the digest, about 1 microgram will appear in the distillate. The importance of this phosphorus interference will obviously depend on the relative amounts of phosphorus and arsenic in the digest. In most biological work it becomes important only when large amounts of urine, or specimens of nervous tissue or bone are to be analyzed. In such cases, phosphorus interference may be eliminated by pouring first the distillate into a second distilling flask, adding 2 to 3 cc. of concentrated nitric acid and 5 cc. of concentrated sulfuric acid, heating down to strong fumes of sulfuric acid, then redistilling. Antimony does not interfere with the determination.

**RATE OF DISTILLATION AND LIMITS OF ACID TOLERANCE.** The rate of distillation of the arsenic under the heating conditions described depends upon the amounts of sulfuric acid and water present. The 4-minute distillation time will allow complete recovery of the arsenic unless the loss in sulfuric acid volume during digestion has been more than 40%.

The amount of hydrobromic acid distilled in the 4-minute period varies between 2.5 and 4.0 milliequivalents. Using the color solutions as described there is no significant difference in the color intensity in the range between 1.0 and 5.0 milliequivalents. There is thus an adequate margin of safety with respect to acidity.

SUMMARY

A rapid method for the determination of small amounts of arsenic in biological material is described in which the arsenic distillate is obtained in pentavalent form. This distillate can be used without further treatment for the final colorimetric determination with ammonium molybdate. The method is rapid, and has given results comparable in accuracy to other published methods for microdetermination of arsenic.

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# Determination of Nitric Oxide Using Solid Reagents

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SMITH and Leighton (5) have described a micromethod for the determination of nitric oxide in mixtures of hydrogen or oxygen which consists in a modification of the macromethod of Baudisch and Klinger (1). Their procedure is based on the oxidation of nitric oxide by the addition of oxygen and the rapid absorption of the resulting oxides of nitrogen by a moist potassium dichromate bead. The method is indirect, because the amount of oxygen required is variable and consequently the amount of hydrogen or nitrogen in the original mixture must be calculated. In analyzing for residual hydrogen after removing all the remaining oxygen by combustion with an excess of hydrogen (a known volume of hydrogen being added if necessary). A further advantage is the possibility of contaminating the mercury surface through the presence of the oxides of nitrogen.

The present method is an adaptation of that of Divers (3), who found that alkaline sodium sulfite solution readily absorbed nitric oxide with the formation of sodium hyponitrososulfate. Further work by Moser and Herzner (4) showed that the reagent is superior to ferrous sulfate in its capacity for absorbing nitric oxide but the rate of absorption was somewhat less.

A pellet of potassium hydroxide is ground in a mortar and sodium sulfite crystals ( $\text{Na}_2\text{SO}_3 \cdot 7\text{H}_2\text{O}$ ) are added till a thick paste is formed. No water is required, as the mixture becomes moist. The paste is then formed into a bead on a platinum loop and if sufficient sodium sulfite has been added very little drying is necessary. If the bead is thoroughly dried no absorption takes place, but aside from this the moisture content does not appear to be critical. When placed in the gas containing nitric oxide absorption is complete in 5 to 10 minutes.

Table I is indicative of the accuracy obtainable by this method, using the Blacet-Leighton apparatus (2).

Table I. Determination of Nitric Oxide

Determination	Volume of Sample Cu. mm.	Nitric Oxide		Difference %
		Theoretical	Determined	
		%	%	
Nitric oxide-hydrogen mixtures				
1	41.68	0.0	0.2	-0.2
2	47.17	13.4	13.5	-0.1
3	51.32	21.6	1.4	0.2
4	65.11	38.4	38.6	-0.2
5	81.18	50.2	50.0	0.2
6	60.83	66.6	66.2	0.4
7	49.12	80.4	80.0	0.4
				Av. 0.3
Nitric oxide-ethylene mixtures				
1	41.97	0.0	0.2	-0.2
2	45.79	8.8	8.4	0.4
3	51.81	19.4	19.0	0.4
4	64.73	35.4	35.0	0.4
5	83.56	49.8	49.7	0.1
6	62.85	65.8	65.0	0.8
				Av. 0.4

In contrast to the method of Smith and Leighton, the present method can be used in the presence of combustible gases other than hydrogen, and very satisfactory results have been obtained in the presence of acetylene as well as ethylene and hydrogen.

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# Colorimetric Determination of Traces of Osmium

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THE work here described was undertaken to develop a colorimetric method for determining minute amounts of osmium after volatilization as the tetroxide, with special reference to the determination of the element in meteoric iron. Goldschmidt and Peters (3) and I. and W. Noddack (4) have determined the abundance of the platinum metals in meteorites by spectrographic methods. From these studies it appears that the average osmium content of the nickel-iron phase of meteorites lies somewhere in the range 3 to 10 parts per million. This order of magnitude is such that osmium can be determined successfully colorimetrically by making use of the sensitive thiourea reaction discovered by Chugaev (1), after isolation of the tetroxide by distillation from a 1- to 2-gram sample.

The procedure ordinarily used for the distillation of decigram (2) and milligram-centigram (6) quantities of osmium tetroxide involves passage of a stream of air through the boiling nitric acid solution and absorption of the tetroxide in several receivers of dilute hydrochloric acid saturated with sulfur dioxide; the solution is then evaporated with hydrochloric acid and osmium precipitated hydrolytically as the hydrous dioxide.

This procedure has been modified for the present purpose. Distillation is made by boiling without passage of air through the solution. Hydrochloric-sulfurous acid is retained as the absorbing solution, but the latter is not evaporated after the distillation, because this results in serious losses of osmium. The osmium in the hydrochloric acid-sulfur dioxide solution reacts readily with thiourea to form the red complex, which is stated (1) to have the composition  $[\text{Os}(\text{NH}_2\text{CSNH}_2)_6]\text{Cl}_3 \cdot \text{H}_2\text{O}$  in the solid state. Since concentration of the absorbing solution by evaporation after the distillation is not admissible, the volume of the distillate must be kept as small as possible to avoid undue loss in sensitivity. Fortunately, osmium tetroxide is readily volatilized, so that boiling off one fifth of the original solution gives a quantitative expulsion of small amounts of osmium. In one experiment, 140 ml. of solution containing 12 micrograms of osmium were distilled according to the procedure described below, and it was found that approximately 70% of the osmium was present in the first 10 ml. of distillate collected and 30% in the second 10-ml. portion; no osmium was detectable in the third 10 ml. of distillate. Usually the solution to be distilled need not have a volume greater than 50 ml., so that only 10 ml. of distillate need be collected. A single portion of hydrochloric acid-sulfur dioxide absorbing solution having a volume of 10 ml. (or even 5 ml.) suffices for satisfactory collection of osmium tetroxide (Table I).

The volume of the final solution in which the color has been developed can be kept down to 15 to 25 ml. With a photoelectric photometer the limit of detectability of osmium is then 1 or 2 micrograms when a layer of solution 1 cm. thick is examined in green light (a solution containing 1 p.p.m. of osmium gives an extinction of ca. 0.015 in 1-cm. depth with a green filter). The use of a visual colorimetric method is less satisfactory than a photometric method because thiourea gives a yellow color with a sulfur dioxide solution. The formation of this yellow substance is of no

importance in a photometric method, since it absorbs green light to a negligible extent (the transmittancy of a blank solution 1 cm. in thickness, under the conditions recommended below 99.9% or more with a Cenco No. 2 green filter).

The reaction between thiourea and osmium in the hydrochloric acid-sulfur dioxide solution is rapid even at room temperature and full color intensity is attained in less than 5 minutes. In preparation of known solutions for the construction of the standard curve, osmium must be added as the tetroxide. Osmium chloroosmate gives no appreciable color with thiourea at room temperature in hydrochloric acid medium, even after several days' standing. The red color appears only on heating (most rapidly if stannous chloride is added). This behavior indicates that osmium is not present to any extent as chloroosmate in the absorbing solution. The osmium-thiourea color system obeys Beer's law.

When an attempt was made to determine osmium in the presence of metallic iron by dissolving the latter in 5 N nitric acid in a distilling flask and then distilling the solution, the results were markedly low. The reason for this was not further investigated but the evolution of nitric oxide is apparently to blame. This difficulty was avoided by dissolving the iron in sulfuric acid, oxidizing the ferrous salt with potassium permanganate, destroying the excess of permanganate and manganese dioxide with a small amount of ferrous salt, then adding nitric acid, and distilling. The excess permanganate and any higher oxides of manganese must be destroyed, else ruthenium will distill as tetroxide with osmium and interfere by giving a blue color with thiourea.

## APPARATUS

There is required an all-glass distilling apparatus consisting of a round-bottomed flask (provided with an inlet tube for addition of reagents) which is connected by means of a ground-glass joint to a water-cooled condenser. The distilling apparatus used in the present work was essentially the same as that described by Robinson, Dudley, Williams, and Byers (5) for distilling selenium and arsenic from soil samples. In this apparatus the thistle tube for addition of reagent solutions is fused into a ground-glass connection, so that it is easy to remove any insoluble material from the flask at the end of the distillation and examine it for osmium. The distilling flask may have a volume of 250 to 500 ml.

Table I. Colorimetric Determination of Osmium after Distillation of Tetroxide

No.	Iron Gram	Addition Ru <sup>III</sup> γ	Os Taken γ	Os Found γ
1	..	..	7.5	7
2	..	..	18.8	17
3	..	..	37.5	36
4	1	..	7.5	7
5	1	..	18.8	18
6	1	..	37.5	34
7	1	25	18.8	18
8	1	50	18.8	17
9	1	50	18.8	18
10	1	25	3.8	3

## SPECIAL SOLUTIONS

OSMIUM TETROXIDE, 0.005% osmium in 0.1 N sulfuric acid. This solution is prepared by dilution of a stronger one, which may be obtained as follows: Make a number of scratches with a file on a 0.5-gram ampoule of osmium tetroxide and weigh the ampoule. Drop the ampoule into a glass-stoppered bottle (200 ml.) containing about 50 ml. of water. Break the ampoule by shaking the bottle, and when the osmium tetroxide has dissolved, decant most of the supernatant liquid into a volumetric flask (250 ml. for example). Rinse the bottle well with successive portions of water and transfer these to the volumetric flask, taking care to leave all the glass fragments in the bottle. Then transfer

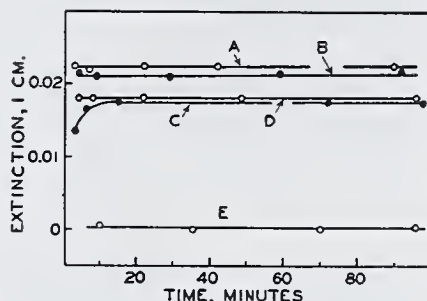


Figure 1. Color Intensity of Osmium-Thiourea Solutions as a Function of the Time of Standing after Addition of Thiourea

Green filter, Cenco No. 2

- A. 1.47 p.p.m. of Os as  $\text{OsO}_4$  in a solution 2 N in HCl and containing 0.2% thiourea; room temperature (27° C.)
- B. As in A except 4 N HCl
- C. As in A except 6 N HCl
- D. 0.5 ml. of  $\text{OsO}_4$  solution containing 0.0368 mg. of Os treated with 10 ml. of 6 N HCl saturated with  $\text{SO}_2$  and allowed to stand at room temperature for 20 minutes; 0.5 ml. of 10% thiourea solution then added and whole diluted to 25 ml. with water.
- E. 2.0 p.p.m. of Os as chloroosmate in 2 N HCl and 0.2% thiourea solution at room temperature.



fragments to weighed filter crucible and obtain the weight of the whole after drying. The weight of the osmium tetroxide is used to prepare the standard solution. The standard solution is obtained by difference.

THIOUREA, 10% aqueous solution.

POTASSIUM PERMANGANATE, 5% solution.

HYDROCHLORIC ACID-SULFUR DIOXIDE SOLUTION, 1:1 hydrochloric acid freshly saturated with sulfur dioxide.

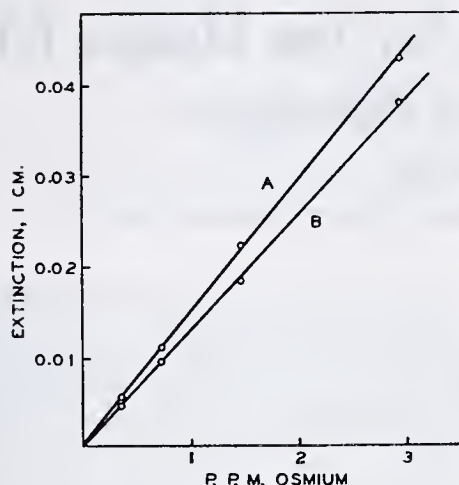


Figure 2. Extinction-Concentration Curves for Osmium Tetroxide-Thiourea Solutions

#### PROCEDURE

The sample solution should have a volume such that it is ready for distillation, after addition of nitric acid and permanganate, the total volume is less than 50 or 60 ml. Chlorides must be absent, and if permanganate oxidation is necessary the solution should be about 1 N in sulfuric acid. Transfer the solution to the distilling flask, and if ferrous iron or other reducing substances are present, add potassium permanganate solution until excess of a drop is present as indicated by the color change; avoid getting permanganate on the neck of the flask. Next add approximately 50 mg. of ferrous ammonium sulfate hexahydrate to destroy permanganate and higher oxides of manganese. The volume of the solution at this point should be 35 to 40 ml. Add a few small grains of pumice, connect the flask to the condenser, and heat the solution slowly to near the boiling point to make it certain that higher manganese oxides have been brought completely into solution. Dip the end of the condenser into 10 ml. of hydrochloric acid-sulfur dioxide solution contained in a 25-ml. graduate, the upper half of which has been cut off (a vial or test tube marked to indicate 20 ml. may be substituted). Add 15 ml. of concentrated nitric acid through the inlet of the flask and distill at such a rate that 10 ml. of distillate is collected in 10 to 15 minutes. Transfer the distillate mixture to a 25-ml. volumetric flask, rinsing the condenser and receiver with a few milliliters of water, add 0.50 ml. of thiourea solution, make up to the mark with water. Determine the transmittancy of the solution after 5 minutes (longer standing does no harm), using green light. In constructing the standard curve use 0, 25, and 50 micrograms of osmium as the tetroxide to distillates obtained from osmium-free nitric acid mixtures as already described.

If the amount of osmium is likely to be less than 10 micrograms, use 5 ml. of hydrochloric acid-sulfur dioxide solution contained in a 25-ml. graduate for collecting 10 ml. of the distillate. Add 0.3 ml. of thiourea solution, read the volume of the solution in the graduate (which has been checked for accuracy), and determine the transmittancy as described above.

#### DETERMINATION OF OSMIUM IN METEORIC IRON

The following procedure was used in determining osmium in the Cañon Diablo siderite.

A 1-gram sample was heated near the boiling point with 10 ml. of 6 N sulfuric acid in an Erlenmeyer flask until there was practically no further action. The solution was decanted from the unattacked sample and reserved. The remainder of the metal was dissolved in 10 ml. of hot 6 N hydrochloric acid. The solution was then treated with 10 ml. of 6 N sulfuric acid and evaporated to fumes of sulfuric acid. The evaporation to fumes was repeated after dissolving the salts in water. The residue was then treated with about 10 ml. of water to bring all but a small amount of insoluble material into solution. This solution and the reserved sulfuric acid solution were transferred to the distilling flask and the ferrous iron was oxidized with permanganate. After

addition of nitric acid, the solution was distilled and osmium was determined as described above (5 ml. of hydrochloric acid-sulfur dioxide solution were used to collect 10 ml. of distillate).

The small amount of insoluble material remaining in the solution after distillation was collected in a small porous porcelain filter crucible, the bottom of which had been covered with a thin layer of quartz powder to facilitate the subsequent removal of the insoluble material. The collected material was dried by washing with acetone, transferred to a nickel crucible, mixed with 1 gram of sodium peroxide, and heated at low redness for 30 minutes. The melt was extracted with 20 ml. of water and the solution heated near the boiling point to decompose peroxide. The solution was transferred to the distilling flask and treated with 10 ml. of 6 N sulfuric acid. Approximately 50 mg. of ferrous ammonium sulfate were added and the solution was heated to destroy nickelic oxide. Nitric acid was then added and the distillation made as already described. No osmium was detected in this distillate.

The osmium content of the Cañon Diablo meteorite thus found is 2.5 p.p.m. Since the method tends to give slightly low results, this value may as well be rounded off to 3 p.p.m. The Noddacks found 3 p.p.m. of osmium in this meteorite, and Goldschmidt and Peters reported an approximate osmium content of 5 p.p.m.

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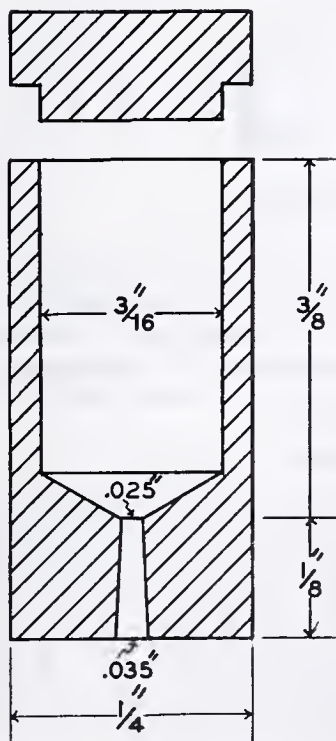
## A Funnel for Filling Capillaries

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FILLING the capillary tubes used for supporting samples in x-ray diffraction cameras is a tedious and time-consuming procedure. The funnel described and illustrated here greatly simplifies this operation. It is constructed of brass, although other materials could be used.

The dimensions of the taper are determined by the size of the capillaries being filled. Plastic capillaries can be wedged into the tapered hole of the funnel firmly enough to stay in place during the filling operation. Glass capillaries must be held in with a slight pressure of the little finger, with the funnel held between the thumb and forefinger. The ground sample is placed in the funnel, and the top edge of the cap is rubbed with a serrated surface such as the side of a pair of tweezers or a dull file in order to shake the powder down into the capillary. If the hole plugs up, a wire can be used to clear it.

Hygroscopic samples can be dried in the funnel in an oven, broken up with a wire, and introduced into the capillary before they have a chance to pick up moisture.





# Carbon Dioxide Generator for the Dumas Method of Determining Nitrogen

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THE all-glass carbon dioxide generators described in the literature (1-5) are essentially identical in principle. They differ chiefly in modifications which affect such features as ruggedness, ease of charging and recharging, and control. All are portable and easily supported. Furthermore, their automatic generating feature is desirable. The carbon dioxide produced is claimed to be very pure, but the actual purity obtained is not mentioned by any of the authors.

A different type of generator constructed in this laboratory, shown schematically in Figure 1, appears to have certain advantages over those referred to above: (1) The generating capacity is over six times as large, over 650 liters of gas, exclusive of the amount wasted during the initial evacuation to remove air impurities; (2) the bicarbonate solution is automatically agitated whenever more gas is generated, and therefore stratification is avoided; (3) any traces of residual air in the acid reservoir are effectively swept out whenever the gas is used; (4) no special safety precautions are necessary during the initial evacuation or later operation; (5) since the gasometer (fitted with a flexible glass extension) is an integral part of the generator the two rubber couplings ordinarily used are eliminated from the train.

system should stopcock *L* be accidentally left open. The 100-ml. mercury-filled gasometer, *G*, is likewise fitted with a small tube, *I*, to prevent air suck-backs in case the three-way stopcock is turned the wrong way when the leveling bulb is lowered to zero the gasometer. All capillary tubing shown is approximately 1 mm. outside diameter by 1.5-mm. bore. Stopcock *L* is used for the initial evacuation, and provides a simple means of obtaining a continuous flow of the gas. The mercury-filled manometer serves to indicate the approximate pressure in the system and also acts as a safety valve. The helix, *K*, made of 3.5-mm. outside diameter tubing, consists of ten loops each about 22 cm. by 2.5 cm. wide. This provides lateral flexibility of over 5 cm. without danger of breakage.

The ball and socket acid valve, *B*, is drawn considerably larger to show details. The outside diameter of the moving part is 7 mm.; hence the bore of joint *F'* must be slightly larger in order to introduce this part safely into position by means of a wire hook. Special attention is called to the loop-shaped delivery tube, *N* (shown enlarged). The trap contains sufficient mercury to seal the capillary tube against back-pressure of height of about 15 cm.; hence the local pressure produced by sudden evolution of the carbon dioxide is prevented from accidentally forcing the bicarbonate solution into the acid delivery tube. The wide-angle funnel-shaped opening at the tip prevents the stoppage of the tube caused by the formation of potassium sulfate crystals. The last bubble of carbon dioxide formed during generation always remains in the funnel tip, thereby effectively separating the capillary column of sulfuric acid above from the bicarbonate solution below.

## CHARGING AND OPERATION

To charge the generator, 3.2 kg. (7 pounds) of potassium bicarbonate are put into the generator bottle, followed by enough water to give a total volume of about 16.5 liters of solution. After the salt has been dissolved by shaking, a rapid stream of tank carbon dioxide is bubbled through the solution for about 3 hours to remove most of the dissolved air. The acid reservoir is filled with 30 *N* sulfuric acid to a point about 1 cm. below the opening of tube *M*. This solution is likewise treated with tank carbon dioxide. The various removable sections terminating with the standard taper joints are then assembled. Joints *A* and *D* are sealed with beeswax, *D*, *D'* with Cenco Plicene cement, and others with Kronig's glass cement. All joints are securely clamped by means of coil springs of suitable strength. The acid valve is then operated to force most of the air out of the acid line. A vacuum pump is attached to stopcock *L* and the system is evacuated until the difference in mercury level in the manometer is about 30 cm. *L* is then closed and enough gas is generated to establish approximately atmospheric pressure. (If any air bubbles remain in the acid line, these can be best removed by opening the acid valve in rapid succession while the system is under reduced pressure.) The evacuation and generation cycle is repeated about 10 times, the pressure is increased until considerable gas blows through the manometer, and the gasometer is filled and completely discharged twice. A rubber tube dipping into a beaker of water is attached to stopcock *L* and the stopcock is opened to permit about 25 ml. of the gas to escape. More gas is again forced through the manometer and the additional steps are likewise repeated. The apparatus should then be in condition for service.

## TEST OF CARBON DIOXIDE PURITY

The generator described above has been used intermittently in this laboratory for over 7 months, and has not been evacuated during this time. Throughout this period, 50- and even 100-ml. samples of the carbon dioxide, tested with the microazotometer, failed to give a readable air blank. On two different days, while the apparatus was in regular use, 500-ml. samples showed 0.4 ml. (4 parts per million) of air impurity in both tests. More recently two additional 500-ml. volumes were tested, the first after the apparatus had stood idle for 3 weeks and the second

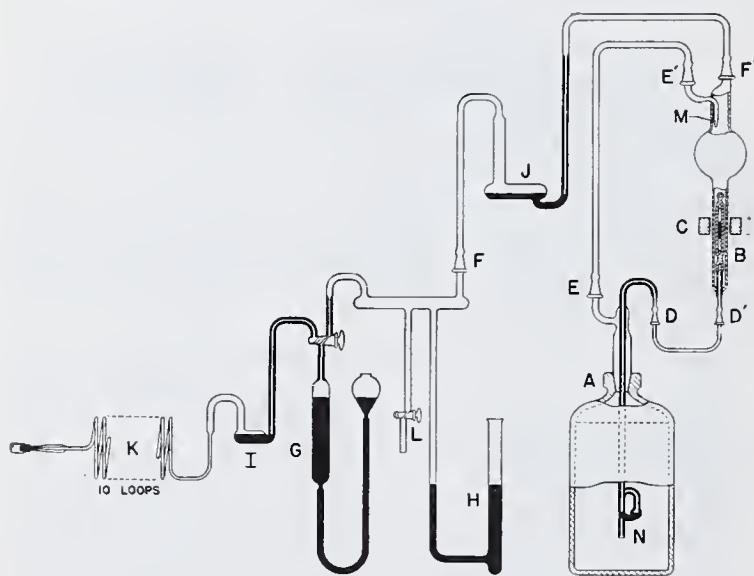


Figure 1. Generator

The chief disadvantage of this generator is that certain parts (particularly the generator bottle, acid reservoir, and gasometer) must be rigidly supported to prevent breakage due to possible misalignment after the parts are assembled. The apparatus is, however, safely portable when mounted on a suitable platform.

## APPARATUS

In Figure 1, the generating chamber is a 19.5-liter bottle with the neck accurately ground to take a 34/45 Pyrex standard taper joint, *A*, which is attached through two 7/25 joints, *D*, *D'*, to the 1-liter capacity acid reservoir. The acid flow is controlled by the magnetic valve, *B*, actuated by the solenoid, *C*, which consists of about 700 turns of 22-gage copper wire, and is operated with a 6-volt battery. The carbon dioxide generated at *N* leaves the bottle through the tube terminating in 10/30 joints, *E*, *E'*, then enters the empty space in the acid reservoir through the vertical tube, *M*, where it sweeps out any air impurities. The mercury trap, *J*, prevents possible suck-back of air into the main



er an additional 5 weeks. Air impurity blanks of 0.002 and 0.03 ml., respectively, were found. In all these tests the generator was connected to the azotometer by means of a short glass connection which was 9 cm. long, but otherwise identical to a regular micro combustion tube. Since it requires about 1 hour to pass 500 ml. of carbon dioxide into the microazotometer, any air which diffuses through the two rubber connections during this time is included in the observed air blank. Hence the actual

purity of the carbon dioxide is probably even higher than the tests indicate.

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## Rapid Digestion Method for Determination of Phosphorus

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A RAPID quantitative method for the determination of phosphorus is always useful in the analytical laboratory. Perchloric acid either alone or in mixtures with other acids has been used to oxidize organic matter previous to the determination of mineral constituents (3, 4). Work in this laboratory has shown that the presence of molybdenum in a perchloric-sulfuric acid mixture markedly increases the rate of oxidation of organic matter.

Most colorimetric methods for the determination of phosphorus are modifications of the Misson (6) or Fiske and Subbarow methods in which molybdenum is a reagent. The use of molybdenum in small amounts as catalyst proved not to interfere with the quantitative colorimetric determination of phosphorus; hence a rapid method was developed for determining phosphorus by the combined techniques of perchloric-sulfuric acid digestion in the presence of molybdenum followed by colorimetric analyses. Results of phosphorus determinations on feeds tested with perchloric-sulfuric acid mixture in the presence of molybdenum as a catalyst are presented here and compared with results using the official ashing method (1).

#### ANALYTICAL PROCEDURE

**DIGESTION MIXTURE.** Dissolve 30 grams of sodium molybdate in 50 ml. of distilled water, then slowly add 150 ml. of concentrated sulfuric acid to the molybdate solution. Allow this solution to cool and then add 200 ml. of 70 to 72% perchloric acid.

**DIGESTION OF ORGANIC MATERIAL.** Transfer not more than a 500-mg. sample to a dry 100-ml. Kjeldahl flask, and add 5 ml. of the digestion mixture and a few glass beads to prevent bumping. Heat the flask slowly over a microburner. Oxidation will be complete in 1 or 2 minutes. At this time the burner may be turned off and the digestion allowed to proceed under its own generation of heat. Wash down any adhering particles on the side of the flask by swirling the flask gently, add 2 ml. of perchloric acid, and heat the flask back on the burner, and heat until the digestion is complete. Digestion is usually complete within 3 or 4 minutes, and the solution is then clear and no charred material remains. Dilute the digestion mixture to a volume of 100 ml. with distilled water. Filter this solution or let it stand to permit any silica to settle out. Take a suitable aliquot for the colorimetric determination, and adjust the acidity with perchloric acid to the approximate range of acidity stated in the method used. Since there is a relatively wide acid range in these methods, the approximate adjustment of perchloric acid concentration can easily be made.

The phosphorus was determined by two different colorimetric methods—the development of a blue color by reducing the phosphomolybdate as described by Sherman (7) and the development of a yellow color by the formation of a phosphovanadomolybdate compound by the method of Koenig and Johnson (5). The percent transmission was determined with the Cenco photometer, using a 420 m $\mu$  filter for the yellow color, 600 m $\mu$  for the blue color, and a reagent blank as a reference liquid. A standard reference curve was made by plotting values of known amounts of phosphorus against the photometer readings on semilogarithmic graph paper.

For routine analysis with samples in which the phosphorus

range is suitable, the Kjeldahl digestion flask may be calibrated to 100 ml. and the reagents added directly to this flask. By the use of the phosphovanadomolybdate method, samples containing 0.05 to 0.5% may be determined directly without further dilution. For materials containing less than 0.1% phosphorus and with a 500-mg. sample the reduced phosphomolybdate method (7) is more suitable, while for material containing higher amounts of phosphorus the vanadate method (5) appears preferable because of the greater stability of the color.

#### DISCUSSION OF RESULTS

Table I shows good agreement of phosphorus in some typical feeds by the perchloric-sulfuric acid digestion method as compared with the longer ashing method. Values are also given as obtained by two previously published colorimetric procedures. Replicate results by the shorter acid digestion method were always as good as by the ashing method.

Several samples have been digested with perchloric-sulfuric acid in the presence of molybdenum, but no explosions have resulted. With a sample of 500 mg. or less, the reaction proceeds smoothly and a set of six samples can be digested in less than 10 minutes.

Table I. Phosphorus Values

Sample	Ashing			Digestion			Difference <sup>c</sup>
	Blue <sup>a</sup> %	Yellow <sup>b</sup> %	Average %	Blue <sup>a</sup> %	Yellow <sup>b</sup> %	Average %	
Clover chaff	0.094	0.090	0.0940	0.095	0.096	0.0955	+1.6
Beet pulp	0.087	0.088	0.0875	0.087	0.092	0.0895	+2.3
Corn silage	0.234	0.225	0.2295	0.220	0.227	0.2235	-2.6
Range grass	0.060	0.060	0.0600	0.060	0.064	0.0620	+3.3
Barley	0.362	0.357	0.3595	0.362	0.357	0.3595	0.0
Corn	0.306	0.312	0.3090	0.309	0.306	0.3075	-0.5
Alfalfa	0.164	0.168	0.1660	0.167	0.170	0.1685	+1.5
Alfalfa	0.191	0.193	0.1920	0.195	0.200	0.1975	+2.9
Alfalfa	0.110	0.106	0.1080	0.104	0.102	0.1030	-4.6
Meat meal	4.560	4.560	4.5600	4.800	4.800	4.8000	+5.3
Soybean	0.554	0.552	0.5530	0.567	0.555	0.5610	+1.4
Fish meal	2.920	2.970	2.9450	3.070	3.100	3.0350	+3.1
Wheat	0.420	0.419	0.4195	0.417	0.417	0.4170	-0.5
Peas	0.480	0.480	0.4800	0.460	0.468	0.4640	-3.3
Poultry ration	0.815	0.800	0.8075	0.803	0.820	0.8115	+0.5

<sup>a</sup> Phosphomolybdate blue, Sherman method (7).

<sup>b</sup> Phosphovanadomolybdate yellow, Koenig and Johnson (5).

<sup>c</sup> Ashing method, basis of 100.

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## All-Glass Midget Impinger Unit

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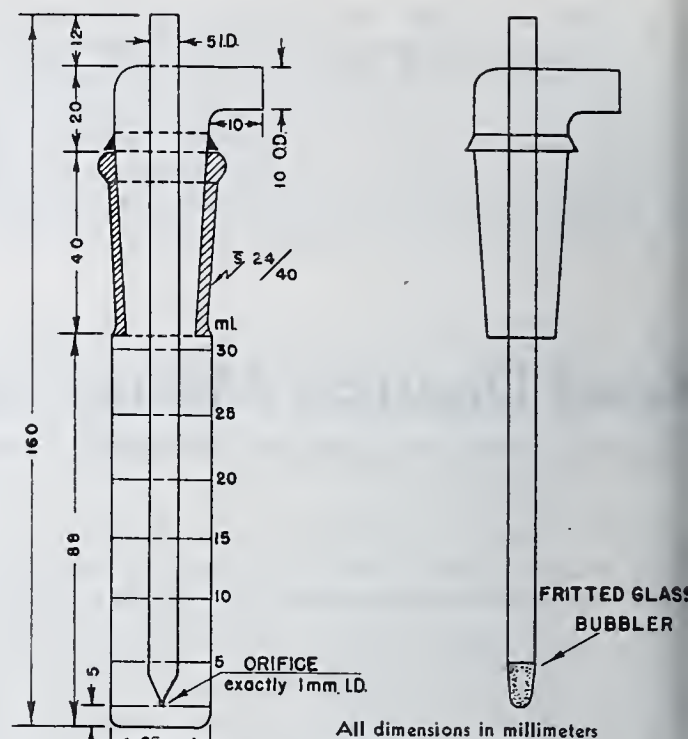
INCREASED use of the midget impinger (2) for collecting various atmospheric contaminants has revealed the following disadvantages:

1. The rubber stopper deteriorates through contact with various acids, alkalis, and solvents used as collecting fluids. It is extremely difficult to clean the rubber stopper when slight deterioration has begun. This may result in contamination of the sample. Certain solvents dissolve a color from the stopper, which interferes in colorimetric analysis.

2. The side arm of the impinger flask is sufficiently low to permit drawing over a portion of various alkalis and solvents used as collecting fluids.

3. When the stopper is placed in the flask, an annular groove is formed where the stopper contacts the flask. Air contaminants may settle in this groove and cause subsequent contamination when the sample is transferred in the field.

To eliminate these disadvantages, an all-glass collecting unit (see diagram), similar to the large impinger unit (1), has been designed and used satisfactorily during the past year. The all-glass equipment can be cleaned thoroughly. The standard taper permits interchanging of parts without readjusting the position of the impinging orifice, which was necessary in the earlier design. The side arm is 138 mm. above the bottom of the flask, as compared to 103 mm. in the earlier design. This added height reduces the possibility of a draw-over when certain solvents and alkalis are used as collecting fluids. A shoulder around the standard taper stopper covers the groove formed between the stopper and the flask, and prevents the settling of dusty material in the groove where it may contaminate the sample during transfer.



For certain purposes, similar all-glass equipment has been designed with a fritted-glass bubbler replacing the impinging orifice. Thus, a single type of unit may be used over a wide range of collecting requirements.

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## A Simple Titration Rack

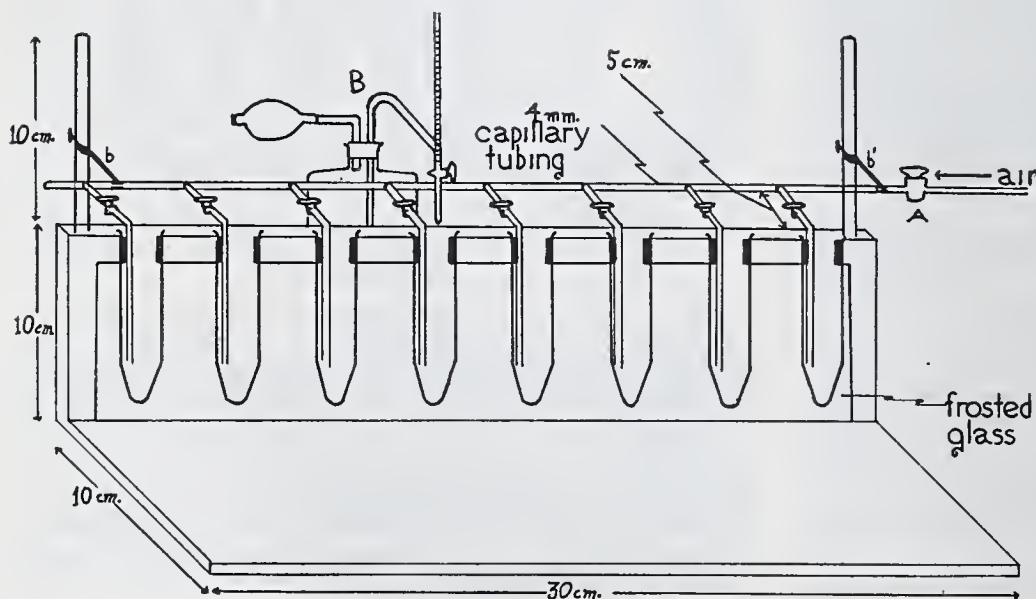
E. C. CANTINO, Division of Plant Nutrition, University of California, Berkeley, Calif.

RECENT investigations in this laboratory have involved the use of various semimicrochemical methods of analysis. Such methods often require centrifugation of minute quantities of material in small conical centrifuge tubes, and subsequently a titrimetric determination of this material in the same vessel. However, adequate agitation during the titration is difficult in

such containers and the process becomes increasingly tedious when a number of determinations are necessary. In order to obviate this difficulty and to provide a rapid method of analysis, a simple titration rack was designed as illustrated.

The stand consists of a wooden base and frame which supports a frosted-glass background. Eight brass spring clips are attached to the frame, so that centrifuge tubes may easily be slipped firmly into position. The glass manifold which serves as an aerating device can then be manipulated into position by means of clamps *b* and *b'*, attached to vertical wooden rods which in turn are supported by the frame.

The rate of air flow is regulated by the control valve, *A*. The small valve above each tube is subsequently opened when the contents are to be titrated. By moving the reagent bottle and attached buret, *B*, consecutively from one tube to the next, a series of eight determinations can be rapidly completed.



### ACKNOWLEDGMENT

The author wishes to thank J. Wells for help with the construction of the apparatus.



# Improved Apparatus for Use in Chromatography

WM. R. CROWELL AND OTTO KÖNIG<sup>1</sup>, University of California, Los Angeles, Calif.

CHROMATOGRAPHIC work the disadvantage of the inaccessibility of the adsorption column while developing the chromatogram is often realized, especially if a so-called colorless chromatogram is in progress. The development is usually followed by pushing the column of adsorbent out of the tube and brushing solutions of suitable reagents along its surface in order to obtain colored reaction products which indicate the zones of various adsorbates (1, 2, 3). In case the chromatogram is fully developed, the entire run must be repeated. If an archchromatogram is developed, the glass of the tube may interfere with the fluorescence phenomenon. In the development of inorganic chromatograms, the developers passed through the adsorbent change entirely by chemical reaction the nature of the components to be resolved.

A tube has been designed which permits one to follow the development of colorless chromatograms by brushing or spotting reagents on the surface of the adsorbent with no loss of time or material, and also to test inorganic chromatograms with traces of reagents, while the bulk of the material remains in the tube in its original state.

The chief feature of the device is a section cut from a small portion of the circumference of the tube along its entire length, forming a tight-fitting lid in the corresponding lengthwise opening. The earliest tubes were of glass, but later ones were of plastic (Lucite). The latter are limited in their use to chromatograms in which only solvents are used which do not attack the plastic, or where a slight attack and presence of dissolved plastic do not affect the tests—e.g., water, alcohol, and ligroin. Metals might prove useful in the construction of such tubes. The tubes used by the authors are 240 mm. long with an inside diameter of 15 mm. They were machined from a 24-mm. round Lucite rod, leaving a wall thickness of 1 mm. Two symmetrical cuts converging at an angle of 90 degrees were made along the entire length of the tube to produce an opening 5 mm. wide on the inside and 15 mm. wide on the outside circumference. From a square-shaped Lucite rod a corresponding circular segment was

machined fitting smoothly into this opening, and after it was set in the tube the inside was machined perfectly smooth. A short section of the lower end of this two-piece tube was machined so as to reduce its diameter somewhat and produce a slight taper, and an adapter with suction tube, made from Lucite, was fitted into it in such a way that it could not slip entirely up the taper. A clearance of about 15 mm. between the end of the tube and the base of the adapter served as a receptacle for cotton. Near the top of the tube a groove was cut around its circumference and a steel position-tension spring was fitted into it. This made possible an exact replacement of the lid after it was removed. Metal bands with screw and nut hold the tube tightly together.

The adapter is supplied with cotton, the tube is assembled and set into the adapter, the whole apparatus is connected to a suction flask by means of a rubber stopper, the tube is filled in the ordinary way (the authors usually employ slurries of adsorbent in a suitable liquid), and the chromatogram is started. Whenever desired, the apparatus is taken from the flask, the adapter is carefully removed, the metal bands are taken off, and the lid is opened to make the necessary tests. If these indicate incomplete development, the lid is reset and the apparatus reassembled for continued development. Even if particles along the exposed surface are removed with the lid, which needs to be lifted only at one end, the position-tension spring will cause these to return to their original location when the lid is replaced. The minute amounts of reagents used in the spotting or brushing tests do not usually interfere in the continuation of further development. The joints of the lid and tube may be made absolutely tight by winding a strip of scotch tape on the outside surface of the tube. This is recommended for the junction of the tube and the adapter.

Tubes have been opened and reclosed as described several times during chromatographic tests without causing caverns and collapsing of the column, although care must be taken to avoid shocks or injuries to the exposed surface of the adsorbent. It was the experience of the authors that if the lid opening was so large as to expose too much adsorbent surface (one half or even less), the lid could not be reset many times without encountering difficulties due to the formation of cavities and channels as a result of too rapid volatilization of the solvent, as well as failure to replace the lid exactly in its original position.

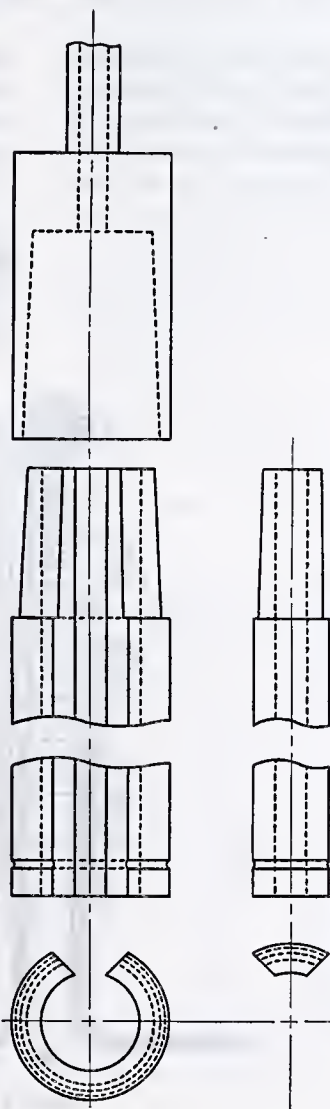
Tubes of square cross section with one side fitted as a removable lid held by screws have also been used.

## ACKNOWLEDGMENT

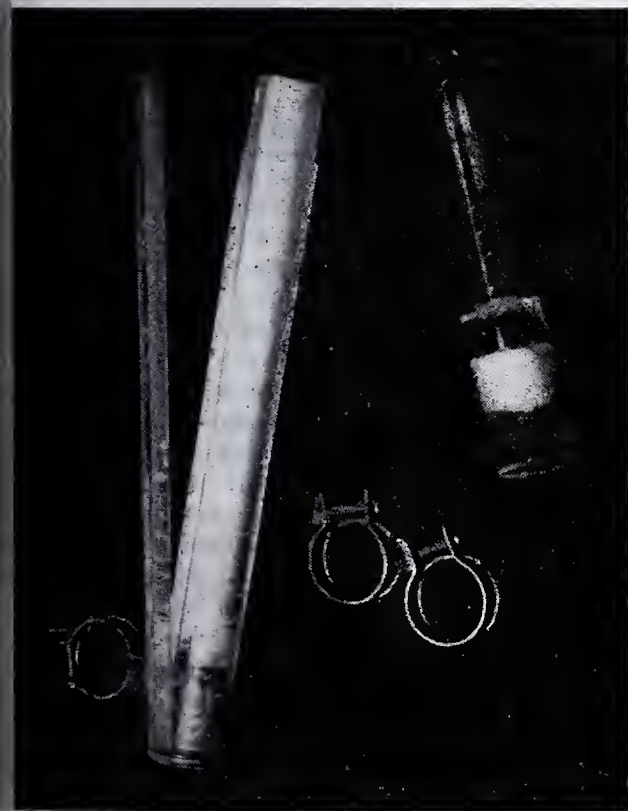
The authors are greatly indebted to W. Thiele, Baroid Sales Division, National Lead Company, for making the tubes and suggesting improvements, especially the position-tension spring.

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Present address, Baroid Sales Division, National Lead Company, Los Angeles, Calif.





# NOTES ON ANALYTICAL PROCEDURES

## Analysis of *n*-Butane–Isobutane Mixtures by the Density Method

ERNEST SOLOMON, The M. W. Kellogg Company, Jersey City, N. J.

A RECENT paper by Leighton and Heldman (1) prompts a brief description of a method that has been employed successfully for several years in this laboratory for the analysis of mixtures of *n*-butane and isobutane. The method is similar to that employed by Leighton and Heldman; however, a description of the apparatus employed may assist other laboratories in assembling a simple and compact analytical unit.

The butane sample, which has been freed of olefins and of lighter and heavier hydrocarbons, is condensed into the inner chamber of a triple-walled Dewar flask. The intermediate chamber, containing liquid propane, is surrounded by an outer evacuated chamber and is further insulated with aluminum foil in which appropriately placed windows have been cut. The temperature of the butane sample is adjusted, by regulating the pressure over the boiling propane, until a small glass float of appropriate density neither rises nor sinks. The pressure over the propane is rapidly adjusted by either applying a pressure of nitrogen or evacuating with a water aspirator through a ballast volume. A small Nichrome heating coil immersed in the propane assists in the rapid attainment of the desired equilibrium tem-

perature; a reflux condenser is provided to return vaporized propane to the intermediate chamber.

It has been found necessary to calibrate for equilibrium flotation temperature with a few known mixtures of *n*- and isobutane since the relationships between isobutane concentration and either propane pressure or sample temperature (expressed as millivolts measured on a multijunction thermocouple immersed in the liquid) are not quite linear. Using calibration charts the method can readily yield results accurate to  $\pm 1\%$  in about 15 to 20 minutes from the time the sample is introduced into the sample chamber until the apparatus is ready for the next sample. About 10 ml. of liquid provide a convenient sample for this technique although there is no reason why this cannot be readily reduced.

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## Fixing Rubber Connections

HERBERT Z. LITTMAN, Research Department, Palestine Potash, Ltd., Jerusalem, Palestine

IT IS general practice to fasten the joint between a glass tube and a rubber tube by means of a small cord, waxed thread, or copper wire. With tubes of small diameter, this method is troublesome and frequently leads to breakage. It is also a disadvantage that the connection cannot be removed without cut-

ting the wire or cord. In certain cases the following method is very convenient, especially if only a small internal pressure prevails and it is desirable to remove and replace the connection frequently:

Cut a small ring, *A*, from a rubber tube of a diameter equal or slightly larger than the tube, *B*, which is to be fixed on the glass tube. Lubricate the ring with glycerol and push it on a cork borer sharpener, *C* (Figure 1, movement 1). Then transfer the ring to a cork borer, *D*, the inner diameter of which is larger than the outer diameter of the rubber tube to be fixed (movement 2). Then slip the end of the rubber tube into the cork borer and transfer the rubber ring onto the rubber tube (movement 3). With the aid of glycerol it is now easy to put the rubber tube with its rubber slip on upon a glass tube, where it will hold tightly.

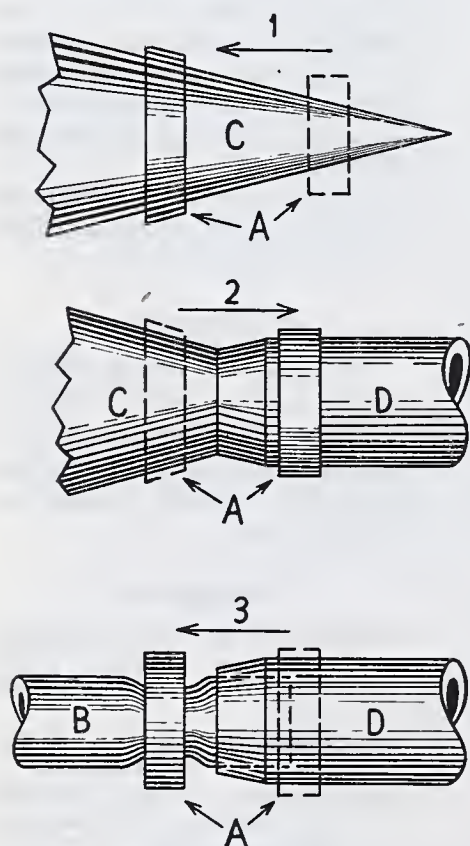


Figure 1

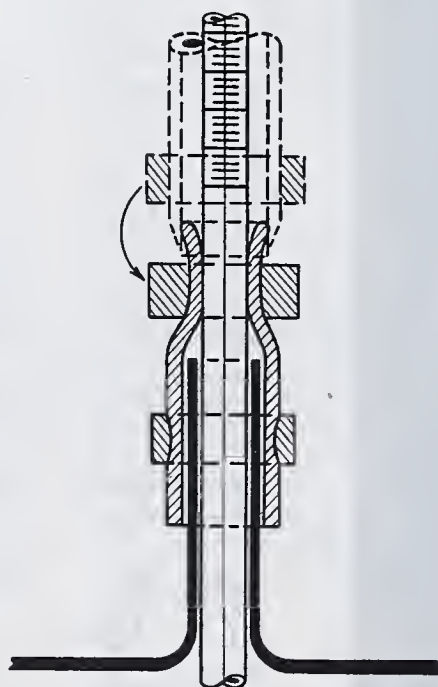


Figure 2

The method is especially useful if a thermometer is to be introduced into an apparatus through a glass tube (Figure 2). Here a wide rubber tube which goes over the glass tube has to be used, and consequently it will be too wide for the thermometer. The connection between the thermometer and rubber tube can be made by a rubber ring cut from a heavy-walled rubber tube. It is easy to slip the rubber ring from the cork borer onto the rubber tube. This should be done after the thermometer is in position, so that no force has to be applied to the thermometer.

Anyone trying the method will quickly learn to make the right choice of diameter, wall thickness, and length of the rings, according to the necessary tightness or ease of connecting and removing.



# Determination of Sulfur in Brass and Bronze by the Combustion Method

ALBERT C. HOLLER AND JAMES P. YEAGER, United States Metal Products Co., Erie, Pa.

THE authors have successfully applied the well-known high-temperature combustion method of Hale and Muehlberg (1) to the determination of sulfur in brass and bronze.

Figure 1 shows the apparatus used for the combustion of the samples. A Dietert-type combustion furnace was used for the analyses. The oxygen was purified by passage through concentrated sulfuric acid, 40% potassium hydroxide, and an Ascarite-calcium chloride bed, in order. The end of the Zirconium combustion tube was packed with about 1.25 cm. (1/2 inch) of ignited asbestos and the temperature was adjusted to the furnace so that the asbestos bed was heated to redness. Chromium sesquioxide was used for the determination of sulfur by combustion in oxygen at 2400° F.) was used and to be the only suitable bedding material for use in combustion boats. Because of the presence of graphite in some of the samples, bromocresol green (pH range 3.8 to 5.4) was used as indicator in the titration of the acid. The sodium hydroxide solution was standardized against a 0.5-gram sample of Bureau of Standards 19c-steel, A.O.H., which contains 0.040% sulfur. A 0.1- to 1.0-gram sample of drillings which remain on a No. 60 mesh pass through a No. 20 sieve was taken for analysis. The sample was burned at 2100° F. in a stream of oxygen which was at the rate of 2 liters per minute. When the combustion

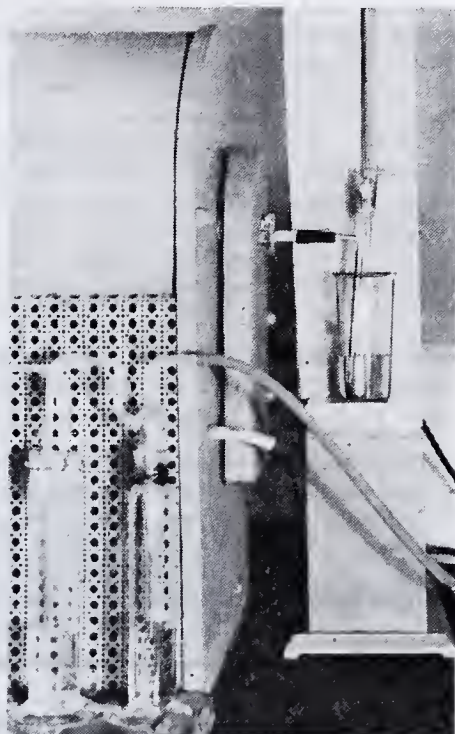


Figure 1

was complete (5 or 10 minutes) the acid was titrated with the standard sodium hydroxide solution with the oxygen still on. The combustion was continued for another 5 minutes and if the acid color of the indicator returned, alkali was added until the end point was again reached.

The method was tested on Bureau of Standards samples 124 of ounce metal and 63a of phosphor bronze bearing metal (Table I).

Table I. Determination of Sulfur

Sulfur present %	Sulfur found %	Deviation %
Bureau of Standards Sample 124, Ounce Metal		
0.071	0.072	+0.001
	0.071	±0.000
	0.074	+0.003
	0.070	-0.001
	0.070	-0.001
	0.069	-0.002
	Av. 0.071	±0.0013
Bureau of Standards Sample 63a, Phosphor Bronze Bearing Metal		
0.11 <sup>a</sup>	0.097	-0.001
	0.098	±0.000
	0.100	+0.002
	0.097	-0.001
	0.100	+0.002
	0.098	±0.000
	Av. 0.098	±0.001

<sup>a</sup> Provisional analysis.

## ACKNOWLEDGMENT

The authors wish to express their deepest appreciation to T. S. Woodward, Carnegie-Illinois Steel Corporation, Youngstown, Ohio, for his helpful comments in connection with this paper.

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# The (Predictable) Concentrating of Standard Solutions Owing to Evaporation

HERMAN A. LIEBHAFSKY, Research Laboratory, General Electric Company, Schenectady, N. Y.

THE author has seen no evaluation of the (predictable) increase in normality caused by the evaporation of water that occurs in a carboy from which a standard solution is being withdrawn and replaced by dry air under the simplest experimental conditions. The problem arose in this laboratory in connection with routine titrations of high precision. In carrying out these titrations, about 40 ml. at a time of carbonate-free sodium hydroxide were forced by means of dry, carbon dioxide-free air from a 15-liter carboy into the buret.

Assume (1) that a carboy of any shape whatever is initially filled with  $V_1$  liters of standard  $N_1$ -normal solution, (2) that each portion of solution withdrawn is replaced by dry air, and (3) that the gas space in the carboy becomes saturated with water vapor between withdrawals. Consider the system when the carboy contains an arbitrarily chosen volume,  $V$ , of  $N$ -normal solution, the solute being nonvolatile. Let 0.024 gram per liter be the water content of saturated air under laboratory conditions. Then, if  $dV$  liter of solution has just been withdrawn, 0.024

$dV$  gram (or ml.) of liquid water will be vaporized, and  $0.024 dV N/1000$  equivalent of solute will be left behind to increase the normality of the remaining solution. But this amount of solute is equal to  $V dN$  equivalents, where  $dN$  is the resulting increase in normality. Upon considering the sign of  $dV$ , equating, transposing, and integrating, one now obtains  $\ln N_2/N_1 = 2.4(10^{-5}) \times \ln V_1/V_2$ , or  $\log N_2/N_1 = 2.4(10^{-5}) \log V_1/V_2$ . (The subscripts refer to arbitrarily chosen initial and final states;  $N$  must increase as  $V$  decreases.)

If the volume of solution in a 15-liter carboy is reduced in the manner prescribed to 1.5 ml.,  $\log N_2 - \log N_1 = 0.0001$ , or the normality of the residuum will increase by 0.02%, which is negligible for most volumetric work.

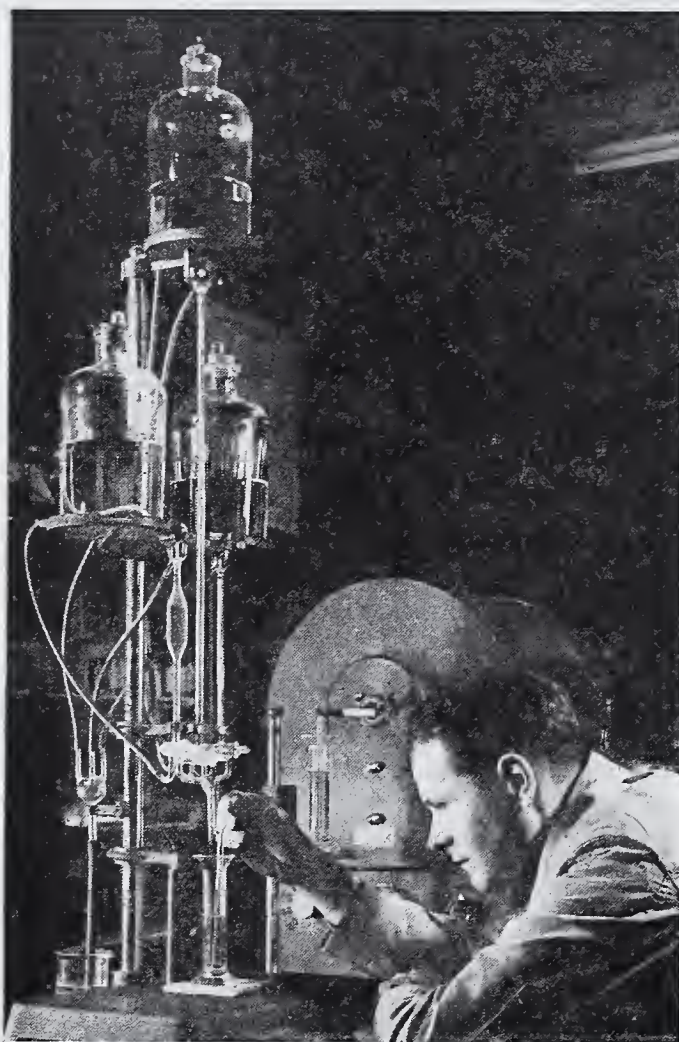
If the simplest experimental conditions do not obtain—if, for example, the room temperature varies markedly, or if there is loss of water vapor from the carboy, as to a drying agent—then unpredictable changes larger than 0.02% may occur in the normality of a standard solution.



## NEW EQUIPMENT

### Sulfur Determinator

The Harry W. Dietert Co., Detroit, Mich., announces an improved 3-minute sulfur determinator for rapid and accurate sulfur determination of steel, iron, nonferrous metals, and materials such as coal and coke.



Sulfur Determinator

The sample is ignited in a high-temperature furnace in an oxygen atmosphere. The sulfur in the sample is converted to sulfur dioxide gas, which is filtered free of all dust particles by a hot ceramic filter within the furnace combustion tube, then bubbled through an alkaline solution which reduces the alkalinity of this solution. The greater the sulfur content in the sample, the lower the alkalinity of the solution. The alkalinity of the solution is measured by titrating with a standard acid solution.

The sulfur determinator is divided into two units: the measuring burets with 2-liter solution bottles, and a stand and support for holding the solution vessel at the furnace. This arrangement increases the speed with which sulfur determination may be made, since one sample may be in process of combustion while another sample is being titrated. The sulfur percentage is read direct from the buret scale.

The gas bubbler is separated from the solution vessel, allowing these parts to be of inexpensive construction and easy to clean.

The large solution bottles hold ample amounts of prepared solutions and may be quickly removed for refilling.

A special blue reflector with a frosted-glass base causes the end point to be a distinct color change from yellow to blue.

## BOOK REVIEWS

**AnalaR Standards for Laboratory Chemicals.** 3rd ed. 230 pages. British Drug Houses, Ltd., and Hopkin & Williams, Ltd., London, 1944.

In this third edition of standards for more than 200 analytical reagent (AnalaR) chemicals many of the tests have been made more delicate or more definite, particularly those for iron. Tests for purities in ferric chloride and ferrous sulfate have been entirely altered, as have those for nickel salts. New tests include: ammonium dihydrogen phosphate, ammonium tartrate, cobalt oxide, nickel acetate, hydrogen peroxide (100 volumes), magnesium acetate, nickel nitrate, perchloric acid (72%), potassium periodate, isopropyl alcohol and sodium dihydrogen phosphate. Four which appeared in earlier editions have been omitted: hydrogen peroxide (10 volumes), perchloric acid (20%), mercuric oxide, and sodium chloride (fused).

**Quantitative Analysis.** Harold Simmons Booth and Vivian Richmond Damerell. 2nd ed. 303 pages. McGraw-Hill Book Co., New York, 1944. Price, \$2.50.

This is a revision of a text intended for use in the elementary quantitative analysis course that normally follows qualitative analysis. The general plan of the book remains the same, but seven determinations have been added: determination of tin in brass, loss on ignition, nitrogen by the Kjeldahl method, antimony in stibnite, sulfur in steel, and a chapter on colorimetric analysis, including determinations of manganese and molybdenum.

### Ceiling Prices on Laboratory Reagent Specialty Solutions

Manufacturers of laboratory reagent specialty solutions containing U. S. tax-paid ethyl alcohol may add to their present ceiling prices the exact amount of the tax in excess of \$4 a proof gallon for the alcohol contained in the solution being sold (Amendment 119 to Revised Supplementary Regulation 14 to General Maximum Price Regulation).

This action, effective April 22, 1944, was taken to correct a correction brought to the attention of OPA by the increased tax on ethyl alcohol which became effective April 1, 1944. Producers of these commercial chemical products, which are solutions of dyes, chemicals or other substances used for scientific and medical research and clinical laboratory uses, are not refunded taxes paid on ethyl alcohol as medicinal and drug manufacturers.

When the Revenue Act of November 1, 1942, increased the tax from \$4 to \$6 per proof gallon on ethyl alcohol, the producers of reagent solutions did not ask for a price adjustment, and assumed that they would be granted a drawback on taxes paid. However, official interpretations of statutes controlling Treasury drawbacks have not permitted recovery of any portion of taxes paid by manufacturers.

On April 1, 1944, the tax was further increased to \$9 a proof gallon which makes it impossible for manufacturers of reagent solutions to continue production under ceiling prices frozen at March, 1942, levels.

Resellers of the solutions may add to their ceiling prices the amount of actual increase resulting to them. The products are sold only to clinics and laboratories, and are not available at retail. Both manufacturers and resellers are required to show the additional charge for the tax as a separate item on invoices.

### A.S.T.M. Committee on Metal Powders

A new standing committee, B-9 on Metal Powders and Metal Powder Products, has been organized by the American Society for Testing Materials to undertake formulation of specifications and methods of tests. W. A. Reich, General Electric Co., is chairman and W. R. Toeplitz, Bound Brook Oil-Less Bearing Co., is secretary.

Three subcommittees have already been organized: Nomenclature and Technical Data, F. N. Rhines, chairman; Metal Powders, D. Noel, chairman; Metal Powder Products, R. P. Koehring, chairman.



## Molecular Weight of Cellulose Measurement of Average Degree of Polymerization

O. A. BATTISTA, American Viscose Corporation, Marcus Hook, Pa.

Viscosity-concentration data are given for five samples of purified cellulose representing the degree of polymerization range from 300 to 3000. On plotting the data on semilogarithmic paper, linear relationships were found to exist, in each case, between (1) the viscosity function  $\frac{\eta_{sp}}{c}$  and concentration, and (2) the relative viscosity function measured at 0.5% concentration and the degrees of polymerization corresponding to values calculated from viscosity-concentration data extrapolated to infinite dilution. The data have been used to derive a mathematical expression by means of which the value of the viscosity function at the standard concentration of 0.5% may be converted to degree of polymerization data equivalent to values obtained by extrapolation of viscosity-concentration data to infinite dilution.

WITH the advent of the more rigorous concepts of cellulose as a long-chain molecule of high molecular weight, the deteriorating action of chemicals and heat on cellulose has come to be considered as a depolymerization reaction whereby the monomeric glucose anhydride units linked continuously in the cellulose chains become severed at irregular intervals in the chains, giving rise to shorter molecules.

The publication of the Staudinger (11, 12, 13) empirical viscosity-molecular weight relationship gave great impetus to the investigation of methods for the determination of the weight-average molecular weights of high polymeric compounds. Copeland (2) has recently reviewed and discussed the more significant papers that have been published relating the viscosity of solutions of high polymers with the degree of polymerization. The procedure most widely used is to relate viscosity data obtained at relatively high concentrations with the value of the viscosity function at infinite dilution through the use of mathematical equations (2, 4, 5, 6, 8, 14).

In this paper, viscosity data are presented for five samples of purified cellulose representing the practical degree of polymerization range from 300 to 3000. These data illustrate that a linear semilogarithmic relationship exists between the relative viscosity measured at 0.5% concentration and the degree of polymerization calculated from viscosity data extrapolated to infinite dilution. The constants of the equation expressing this experimentally determined relationship have been obtained by graphical analysis of the data, and using these constants the equation has been satisfactorily checked against extrapolated values.

### EXPERIMENTAL

**METHOD OF FLUIDITY MEASUREMENT.** The general procedure used for the measurement of fluidity was based on the papers of Clibbens and Geake (1) and Mease (9). The viscometer's design, complete dimensional specifications, method of calibration,

and a discussion of the precision of viscosity measurement obtainable with this type of capillary viscometer, are given in the foregoing papers.

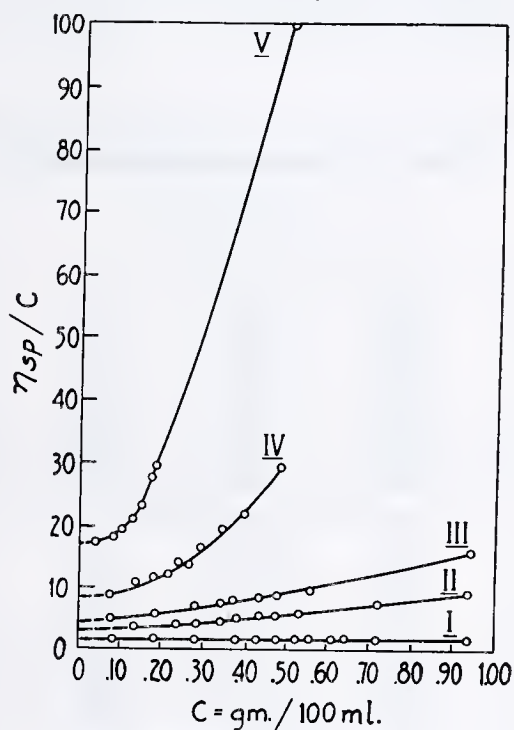


Figure 1.  $\frac{\eta_{sp}}{c}$  vs.  $c$  (ordinary graph paper)

- I. Typical viscose rayon
- II. Low-viscosity rayon wood pulp
- III. Normal-viscosity rayon wood pulp
- IV. Absorbent cotton
- V. Raw cotton

The viscometers used in this work were equipped with ground-glass connections (8, 9) and glass stopcocks. Outside dissolving tubes (10), whereby the viscometers are reserved for the measurement of fluidity, were used.

It was found advisable, in determining the viscosity of high-fluidity celluloselike rayon, to use viscometers possessing capillaries of smaller inside diameter than the 0.88-mm. inside diameter capillary recommended for use with cotton solutions. The large kinetic energy correction that would otherwise be necessary for high-fluidity cellulose solutions may be satisfactorily reduced by the use of viscometers whose capillaries have an inside diameter of 0.675 mm.

Pure copper gauze (80-mesh) was used in the preparation of the cuprammonium solvent. The copper gauze was wrapped around an inlet tube equipped with a fritted-glass jet of D porosity, and maintained below the level of the ammonium hydroxide in the generating chamber. Agitation was provided for by the fritted-glass jet which served to break up the incoming ammonia-laden air into small bubbles. The use of fine-mesh copper gauze facilitated the solution of the copper, and obviated any necessity for filtering the solvent at any time in the process.



of its preparation. A siphon was used to transfer the solvent from the generating chamber to the stock bottle.

The copper content was determined by means of a calibrated photoelectric colorimeter. This method is rapid and was shown to be as accurate as the volumetric method for determining copper. The copper content was adjusted to 15.0 ( $\pm 0.10$ ) grams of copper per liter.

The ammonia content was determined volumetrically and was maintained at 200 ( $\pm 5$ ) grams of ammonia per liter.

The nitrous acid content was determined by means of a Lunge nitrometer and was never found to exceed the maximum limit of 0.5 gram per 100 ml. of solvent.

The solvent was stored under oxygen-free nitrogen at 5° C., and its viscosity in centipoises ranged from 1.32 to 1.36 at 20° C.

All samples of cellulose used in this study received a mild alkaline scouring treatment (1% sodium hydroxide at 40° C. for at least 40 minutes), and a thorough extraction with water and organic solvents. Samples were conditioned at 58% relative humidity and 21.11° C. (70° F.) for at least 24 hours, after which moisture determinations were made in duplicate on each sample. The weight of the sample used for the measurement of fluidity was calculated on a bone-dry basis.

Black glazed analytical weighing paper was used for weighing the samples.

The samples were put up in a constant-temperature room (18° C.), and left on the rotating wheel at this temperature overnight. An oxygen-free nitrogen atmosphere was maintained above the surface of the solvent as it was discharged into the dissolving tubes.

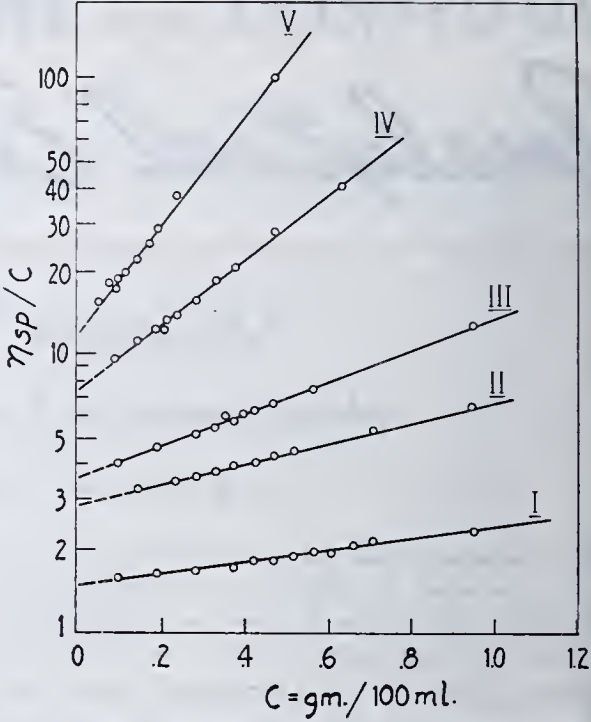


Figure 2.  $\frac{\eta_{sp}}{c}$  vs.  $c$  (semilogarithmic graph paper)

- I. Typical viscose rayon
- II. Low-viscosity rayon wood pulp
- III. Normal-viscosity rayon wood pulp
- IV. Absorbent cotton
- V. Raw cotton

Table I. Viscosity-Concentration Data

$c'$ %	$c$ G./100 ml.	Average Fluidity <i>Rhes</i> at 20° C.		$\eta_{sp}$	$\frac{\eta_{sp}}{c}$
Typical Viscose Rayon					
1.00	0.944	23.3	2.14	2.26	
0.75	0.708	29.1	1.51	2.13	
0.70	0.660	30.8	1.42	2.15	
0.65	0.613	33.5	1.18	1.90	
0.60	0.566	34.8	1.10	1.94	
0.55	0.519	37.0	0.97	1.86	
0.50	0.472	40.0	0.86	1.82	
0.45	0.425	40.9	0.78	1.83	
0.40	0.377	44.9	0.64	1.69	
0.30	0.283	49.6	0.47	1.66	
0.20	0.188	55.7	0.31	1.64	
0.10	0.094	63.5	0.15	1.59	
Low-Viscosity Rayon Wood Pulp					
1.00	0.944	10.2	6.18	6.54	
0.75	0.708	15.3	3.80	5.36	
0.55	0.519	21.7	2.38	4.58	
0.50	0.472	24.0	2.06	4.36	
0.45	0.425	27.2	1.70	4.00	
0.40	0.377	29.8	1.47	3.89	
0.35	0.330	32.9	1.23	3.72	
0.30	0.283	36.3	1.03	3.63	
0.25	0.236	40.0	0.84	3.55	
0.15	0.141	49.9	0.47	3.33	
Normal-Viscosity Rayon Wood Pulp					
1.00	0.944	5.56	12.2	12.81	
0.60	0.566	14.0	4.24	7.49	
0.50	0.472	17.5	3.18	6.73	
0.45	0.425	19.6	2.76	6.40	
0.42	0.395	21.3	2.45	6.18	
0.40	0.377	22.8	2.22	5.88	
0.38	0.358	22.9	2.21	6.17	
0.35	0.330	26.5	1.78	5.39	
0.30	0.283	30.4	1.42	5.02	
0.20	0.188	39.9	0.87	4.62	
0.10	0.094	52.9	0.39	4.14	
Absorbent Cotton					
0.50	0.472	5.19	13.3	28.17	
0.40	0.377	8.28	7.88	20.90	
0.35	0.330	10.3	6.14	18.60	
0.30	0.282	13.6	4.43	15.75	
0.25	0.236	17.4	3.24	13.72	
0.23	0.217	19.1	2.91	13.40	
0.22	0.208	21.0	2.55	12.25	
0.20	0.188	22.5	2.33	12.30	
0.15	0.141	28.5	1.62	11.50	
0.10	0.094	38.8	0.925	9.78	
Raw Cotton					
0.50	0.472	1.45	48.0	101.6	
0.25	0.236	7.38	8.98	38.05	
0.20	0.188	11.4	5.42	28.61	
0.18	0.169	13.9	4.28	25.32	
0.15	0.141	18.3	3.03	21.49	
0.12	0.113	22.6	2.23	19.72	
0.11	0.104	24.9	1.95	18.75	
0.10	0.094	27.1	1.75	18.61	
0.09	0.085	29.8	1.50	17.65	
0.08	0.075	31.5	1.37	18.35	
0.05	0.047	43.2	0.73	15.55	

Pure copper agitators in the form of spirals or solid rods, depending on the viscosity of the sample being tested, were used to minimize the degradative action of oxygen on cellulose in cuprammonium solution (3).

Fluidities were measured at 20° ( $\pm 0.10^\circ$ ) C., and flow times were determined by means of a split-second electric stop clock with an average reproducibility to within less than 1%. The average deviation in the fluidities of the duplicate measurements on a given sample never exceeded 5%, and was usually less than 2%.

A solvent blank was run in duplicate with each series of terminations. A standard sample of cellulose was run as a check blank periodically. New batches of solvent were prepared every 2 or 3 months and 3 liters were prepared at a time.

RESULTS

In Table I viscosity-concentration data are given for each of the five samples of cellulose studied: a typical viscose rayon, a low-viscosity rayon wood pulp, a normal-viscosity rayon wood pulp, absorbent cotton, and raw cotton.

In Table II, degree of polymerization data calculated from infinite dilution values of the viscosity function and using the Kraemer relationship (7), are compared with degree of polymerization data calculated on the basis of the value of the viscosity function at 0.5% concentration.

RELATIONSHIP BETWEEN APPARENT AND BASIC DEGREE OF POLYMERIZATION. It is routine practice in many laboratories to determine the viscosity (or fluidity) of a solution of cellulose in cuprammonium solvent at a standard concentration high enough to make the viscosity measurement as simple as possible. In this way, it is practical to determine relative changes in viscosity and thereby obtain a measure of the degree of depolymerization of cellulose. The arbitrary standard concentrations most widely used are 0.50 and 1.0%, respectively.

The data presented in this paper correlate the values of the viscosity function obtained at the standard concentration of 0.50% (apparent D.P.) for five representative samples of cellulose with the values for the respective viscosity functions obtained at infinite dilution (basic D.P.):

$$\text{Limit} \left( \frac{\eta_{sp}}{c} \right) c \rightarrow 0$$



On plotting the  $\frac{\eta_{sp}}{c}$  vs.  $c$  data for each sample on ordinary graph paper, the curves in Figure 1 are obtained, in which the rate of increase of the slope is dependent upon the degree of polymerization of the sample being studied. Furthermore, as the degree of polymerization increases, the variation in the slope of the curves at very low concentrations precludes reliable extrapolation to the  $\frac{\eta_{sp}}{c}$  vs.  $c$  data. However, when the same data are plotted on semilogarithmic paper, linear curves are obtained for each sample (Figure 2) and more reliable extrapolation is possible. The data have been used to draw a conversion graph (Figure 3) in which the calculated "apparent D.P." obtained for each sample at 0.5% concentration are plotted against the corresponding "basic D.P." calculated from the values of the respective viscosity functions at infinite dilution, using the Kraemer relationship (7).

A more applicable conversion relationship has been obtained by plotting the values of  $(\eta_r + 1)$ , determined from the solution viscosity at 0.5% concentration, against the corresponding values of the "basic D.P." on semilogarithmic graph paper. When this is done, a linear relationship is obtained and is expressed by:

$$\text{Basic D.P.} = a[\log (\eta_r + 1) - b] \quad (1)$$

where  $a$  and  $b$  are constants representing the slope and intercept, respectively, of Figure 4.

The values of constants  $a$  and  $b$  were obtained graphically from Figure 4, and on substituting them in Equation 1 we obtain:

$$\text{Basic D.P.} = 2160 [\log (\eta_r + 1) - 0.267] \quad (2)$$

The Kraemer relationship and constant (7) for cellulose in ammonium solution used to calculate degree of polymeriza-

Table II. Comparison of Degree of Polymerization Data Obtained by Two Methods of Calculation

Material	Degree of Polymerization	
	From infinite dilution data using Equation 3	From data at 0.5% concentration using Equation 2
lignin	3120	3100
lignin	1950	1980
lignin	960	965
lignin	750	735
lignin	390	408

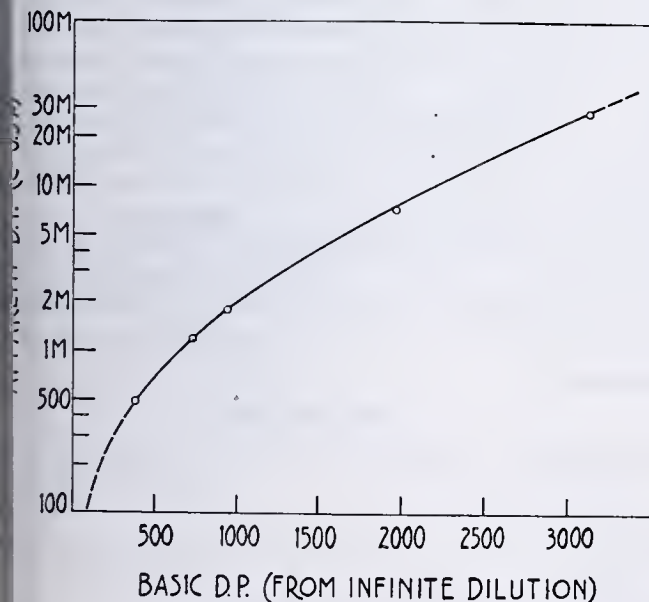


Figure 3. Conversion Graph

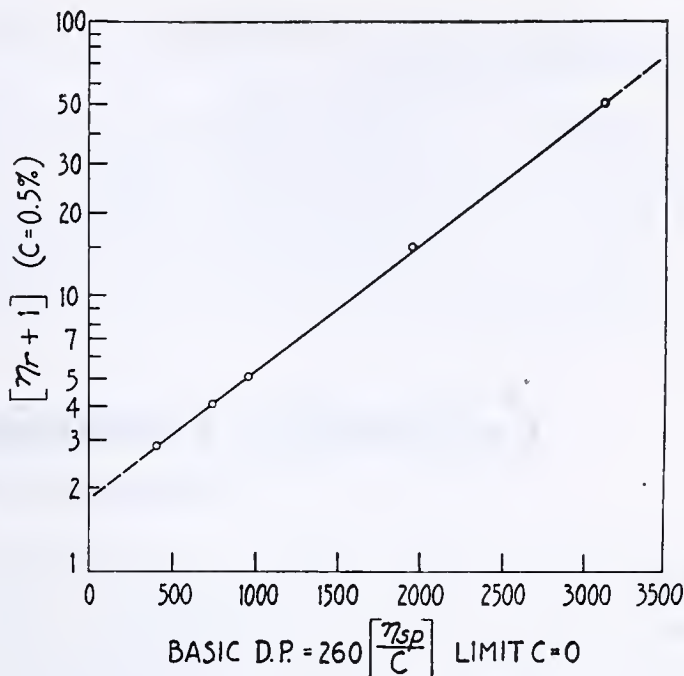


Figure 4. Conversion Relationship

tion data from values of the viscosity function at infinite dilution are given in Equation 3:

$$\text{D.P.} = 260 [\eta] \quad (3)$$

where  $[\eta]$  is the value for the intrinsic viscosity at infinite dilution

$$\text{Limit} \left( \frac{\ln \eta_r}{c} \right) c \rightarrow 0$$

The calculated basic degree of polymerization values obtained using the conversion relationship of Equation 2 are compared in Table II with the values obtained by extrapolation of the viscosity data to infinite dilution and using Equation 3.

## CONCLUSIONS

When viscosity-concentration data, obtained for five representative samples of purified cellulose covering the degree of polymerization range from 300 to 3000, are plotted on semilogarithmic paper, linear relationships are obtained in each case. This permits more accurate extrapolation of the viscosity data to obtain the intercept values of the viscosity function—i.e., values at infinite dilution—from which degree of polymerization data may be calculated.

The logarithmic relationship between the values for the viscosity function  $(\eta_r + 1)$ , obtained at 0.5% concentration, and the respective degree of polymerization data calculated from the values of viscosity function at infinite dilution, is also linear and is expressed by Equation 2. The numerical constants of this equation were obtained by a graphical analysis of the data.

A conversion graph has been drawn relating the "apparent" degree of polymerization obtained at 0.5% concentration to the corresponding values for the degree of polymerization obtained by extrapolation to infinite dilution (Figure 3).

Equation 2 may be used for accurately converting values of the viscosity function obtained at the standard concentration of 0.50% to basic degree of polymerization data equivalent to values obtained by extrapolation of viscosity-concentration data to infinite dilution and using the Kraemer relationship and constant (7).

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## Colorimetric Determination of Nickel in Bronze

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MANY bronzes contain up to 1% of nickel. For these relatively small amounts it would appear that a colorimetric method might be satisfactory for routine work. Feigl (2) found that lead dioxide oxidized nickel in alkaline solution to a valence higher than 2, and that addition of dimethylglyoxime to this solution gave a red coloration rather than a precipitate. This procedure was improved by Rollet (4), who used bromine water instead of lead dioxide, and this method has found many applications (1, 3, 5). This reaction has been applied to the determination of nickel in bronze using a filter photometer such as the Cenco photometer with a cell 10 mm. thick, taking about 17 ml. of solution.

#### PROCEDURE

After the tin is removed by filtration as metastannic acid and the copper and lead by electrolysis, the remaining solution is diluted to 150 ml. and mixed. One milliliter is transferred by pipet to a 100-ml. tall-form beaker, 25 ml. of distilled water are added, and the mixture is shaken after addition of one drop of saturated bromine water. Seven drops of an ammoniacal solution of dimethylglyoxime (10 grams of dimethylglyoxime dissolved in 650 ml. of ammonium hydroxide and diluted to 1 liter) are added and the mixture is again shaken well. The orange-red color develops in alkaline solution immediately on shaking. The solution is transferred to a photometer cell and the absorption determined with the use of the Cenco dark blue filter or a Corning blue filter such as No. 556. The maximum absorption occurs at 475 m $\mu$ . The per cent nickel is obtained from the usual type of straight-line curve plotted on semilog paper. The calibration data for this curve can be obtained through the use of a solution of a c.p. nickel salt standardized gravimetrically, or preferably by removing an aliquot from the regular sample, obtaining the colorimetric value from this aliquot, and using the remainder for a gravimetric determination. With bronzes containing manganese, iron, or aluminum, 3 to 5 drops of a solution of ammonium citrate (25 grams of ammonium citrate dissolved in 30 ml. of water) are added before addition of bromine water.

#### RESULTS AND DISCUSSION

Some typical single results obtained by this method are shown in Table I. In general, it is believed the results are satisfactory for the usual type of bronze. The use of ammonium citrate does not eliminate the interference of manganese and iron but reduces it considerably. The precision and accuracy of this method in the range indicated are 0.02 to 0.04% nickel.

To obtain satisfactory results with this method it is necessary to standardize on a procedure and use it for all determinations. Among the factors which can affect the intensity of the color are time of standing, amount of bromine used, amount of ammonium citrate used, shaking, and temperature. The color intensity increases on standing, the increase being greatest during the first 20 minutes, and tends to level off after 2 hours. A typical increase during the first 20 minutes would be from 0.58 to 0.60% nickel. The use of more than one drop of bromine water and the use of ammonium citrate tend to lower the color intensity slightly—for example, standard 52a gave 0.75% nickel with 1

drop of bromine water, 0.74% with 2 drops, and 0.71% with 3 drops. An approximately equal reduction in values for nickel obtained with 3 to 5 drops of the ammonium citrate solution so that 5 drops of bromine water and 5 drops of the ammonium citrate solution give values of 0.67 to 0.68% nickel for sample. Low results will also be obtained by the use of too small a drop of bromine water, in which case some nickel will be precipitated.

Table I. Nickel Determinations on Bureau of Standards Samples

Sample No.	Interfering Elements %	Nickel	
		Gravimetric %	Colorimetric %
37C	Fe, 0.17	0.58	0.59
37C	Fe, 0.17	0.58	0.57 <sup>a</sup>
37B	Fe, 0.21	0.45	0.46 <sup>a</sup>
37B	Fe, 0.21	0.45	0.46 <sup>a</sup>
52	Fe, 0.12	0.13	0.13
52	Fe, 0.12	0.13	0.13 <sup>a</sup>
124	Fe, 0.38	0.45	0.47
124	Fe, 0.38	0.45	0.46 <sup>a</sup>
52a	Fe, 0.05; Mn, 0.02	0.73	0.75
62	Fe, 1.13; Mn, 1.59; Al, 1.13	0.64	0.70 <sup>a</sup>
Manganese bronze <sup>c</sup>	Fe, 2.2; Mn, 3.1; Al, 3.8	0.00	0.10 <sup>b</sup>

<sup>a</sup> Using 3 drops of ammonium citrate solution, 25 grams per 30 ml.

<sup>b</sup> Using 5 drops of ammonium citrate solution, 25 grams per 30 ml.

<sup>c</sup> A commercial sample.

Some experiments indicate that fairly vigorous shaking is necessary to develop the maximum color intensity, although the values obtained were indecisive. Temperature has little effect on the color, except that a hot solution will give a precipitate rather than a color. Temperatures somewhat above or below room temperature gave substantially the same values.

To reduce the interference of iron and manganese, ammonium citrate may be added. Under these conditions, these elements will give a yellow solution. A proper choice of wave length may serve to eliminate this interference. However, with a blue filter the interference due to iron was found to be about 0.02% nickel for 1% iron, and 0.03% nickel for 1% manganese with the use of 3 drops of an ammonium citrate solution containing 25 grams of the salt in 30 ml. of water. The dark green Corning filter No. 556 reduced the interference somewhat but gave a less satisfactory calibration curve. Copper and zinc in the amounts usually present offer no interference.

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# Increase in Concentration of Insecticide in Freon-12

## Accompanying Transfer or Discharge of an Aerosol-Producing Solution

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he transfer or discharge of solutions in liquefied gases used for the production of insecticidal aerosols, a concentrating effect occurs because of escape of solvent from the solution to maintain the high or density. A mathematical treatment of this effect is given, experiments are described by which it was confirmed for solutions in Freon-12. In that case a discharge of 90% of the liquid raises the concentration of the remaining solution by 8% of value.

HE insecticidal aerosol, produced when a solution of insecticides in a liquefied gas is released into the air (3), met an urgent military need, especially for disinfecting planes and for overseas use. The combination of pyrethrum and sesame oil in dichlorodifluoromethane (Freon-12) produces a very effective nonflammable insecticide that is nontoxic to man and animals. In the manufacture and packaging of this solution, main questions have arisen concerning the physical properties of solutions in liquefied gases. A mathematical treatment of one of these problems, which has to do with change in concentration due to transfer or discharge of insecticide, and confirmatory experimental results are presented in this paper.

At 80° F. (26.7° C.) the density of saturated Freon-12 vapor is 0.0377 gram per cc. As liquid is withdrawn from an aerosol container during use, appreciable quantities of Freon evaporate from the solution remaining in the container to maintain this high or concentration in the increasing space not occupied by the liquid. As a result the concentration of the remaining solution gradually increases as the container empties. While this change is less serious than if the solution progressively weakened, still the need for conservation of insecticide suggests that some consideration be given to the matter, especially in connection with packaging the solution.

### MATHEMATICAL DEVELOPMENT

An approximate estimate of the magnitude of this effect at any fixed temperature can be obtained by means of the calculus, it is assumed that the densities of both gaseous and liquid phases remain constant. This is obviously not strictly true, but it will be shown later that the departure from exactness is inconsequential. The mathematical development follows:

$V$  = volume of container, in cubic centimeters  
 $M$  = weight of initial total content, in grams  
 $M_s$  = weight of initial liquid content, in grams  
 $C$  = initial concentration of insecticide in the liquid, in grams per gram  
 $W$  = initial weight of insecticide in the liquid, in grams  
 $D_s$  = density of insecticide solution, in grams per cubic centimeter  
 $D_g$  = density of solvent vapor, in grams per cubic centimeter  
 $r$  = the ratio  $D_g/D_s$   
 $Q$  = weight of liquid withdrawn (no vapor being allowed to escape)  
 $m$  = weight of total contents after withdrawing  $Q$ , in grams  
 $m_s$  = weight of liquid contents after withdrawing  $Q$ , in grams  
 $c$  = concentration of insecticide after withdrawing  $Q$ , in grams per gram  
 $w$  = weight of insecticide in container after withdrawing  $Q$ , in grams

At any stage of emptying, the weight of insecticide in the container is  $w$ . Withdrawal of an additional infinitesimal weight,

$dm$ , consisting of solution only will cause a corresponding change in the value of  $w$ , as given by the equation

$$dw = c dm = \frac{w}{m_s} dm \quad (1)$$

$$\text{But} \quad \frac{m_s}{D_s} + \frac{m - m_s}{D_g} = V$$

$$\text{Whence} \quad m_s = \frac{VD_s D_g - m D_s}{D_g - D_s} = \frac{m - VD_g}{1 - r} \quad (2)$$

$$\text{and } dm = (1 - r) dm_s \quad (3)$$

$$\text{From 1 and 3} \quad \frac{dw}{w} = \frac{(1 - r) dm_s}{m_s}$$

$$\text{Proceeding to definite integrals,} \quad \ln \frac{w}{W} = \ln \left( \frac{m_s}{M_s} \right)^{1-r}$$

$$\text{Therefore, since} \quad w = m_s c \quad \text{and} \quad W = M_s C$$

$$\ln \frac{m_s c}{M_s C} = \ln \left( \frac{m_s}{M_s} \right)^{1-r}$$

$$\text{from which} \quad \frac{c}{C} = \frac{M_s}{m_s} \times \frac{m_s^{1-r}}{M_s^{1-r}} = \left( \frac{m_s}{M_s} \right)^{-r}$$

If  $M_s$  is known, as it was in some of these laboratory experiments because of the manner of filling,  $m_s$  at any stage is calculable from it and the weight,  $Q$ , of liquid withdrawn, for from Equation 2

$$m_s = \frac{M - Q - VD_g}{1 - r} = M_s - \frac{Q}{1 - r}$$

$$\text{and hence} \quad \frac{c}{C} = \left[ 1 - \frac{Q}{(1 - r) M_s} \right]^{-r} \quad (4)$$

By the aid of values calculated from this equation, the percentage increase in concentration,  $100 \frac{c - C}{C}$ , can be plotted

against  $\frac{100 Q}{M_s}$ , the percentage of solution withdrawn, as is illustrated in Figure 1 for a solution containing 5% of sesame oil in Freon-12. For the construction of this graph  $D_g$  was taken as 0.0377 gram per cc.,  $D_s$  as 1.291 grams per cc., and  $r$  therefore as 0.0292. It shows that a quantity of liquid equal to 22% of the original liquid content must be withdrawn before the concentration of insecticide rises 1%, that removal of 80% causes a 5% rise, and that 92.5% delivery gives only a 9% rise.

If, as will more often be the case, the total content of the container,  $M$ , rather than the liquid content,  $M_s$ , is known, Equation 4 can be converted, because of the relationship

$$M_s = \frac{M - VD_g}{1 - r}$$

into the equivalent form

$$\frac{c}{C} = \left( 1 - \frac{Q}{M - VD_g} \right)^{-r}$$

and  $100 \frac{c - C}{C}$  can be plotted against  $\frac{100 Q}{M}$ , the percentage of total contents removed.

### EXPERIMENTAL VERIFICATION

The errors due to the assumption that the gas and liquid densities are constant can be judged by a consideration of the possible departures of those values from constancy. The possible changes in gas density were derived from measurements of the lowering



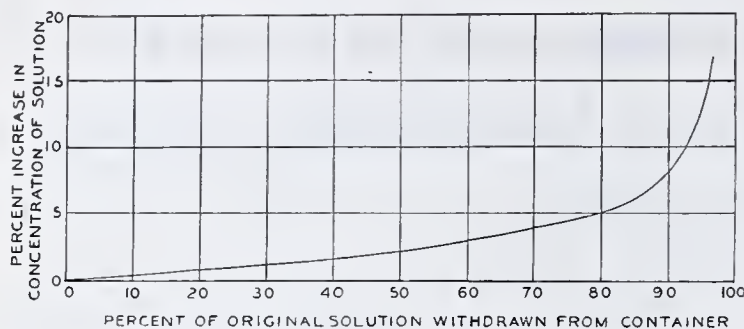


Figure 1. Increase in Concentration of Freon Solution of Oily Insecticide as Contents Are Withdrawn from Container

Table I. Lowering of Vapor Pressure of Freon-12 by Sesame Oil

Sesame Oil %	Vapor Pressure Lowering Mm. of Hg
2.5	6, 7, av. 6.5
5.0	18, 19, 18, av. 18.3
10	23, 24, 28, av. 25
15	35, 37, av. 36

of vapor pressure produced by dissolving various proportions of sesame oil in Freon-12.

The apparatus used to make these measurements (Figure 2) consists of two identical containers with valves connected through a U-tube containing mercury, which acts as a differential manometer. Two small petcocks, one on each side of the manometer, are necessary to operate the apparatus.

Three hundred grams of liquid Freon-12 were always placed in the container on the right and an equal weight of solution was made up in the one on the left. The connections were made to the manometer, and the vapors from the two containers were allowed to enter the manometer simultaneously until the total vapor pressure on each side was exerted. The whole apparatus was then submerged in a transparent water bath. Readings were made at 80° F. after the system had reached equilibrium.

The Freon-12 used contained some nonliquefiable gases, which interfered somewhat. To overcome this interference, containers of the same size with the same volumes occupied by the liquid on

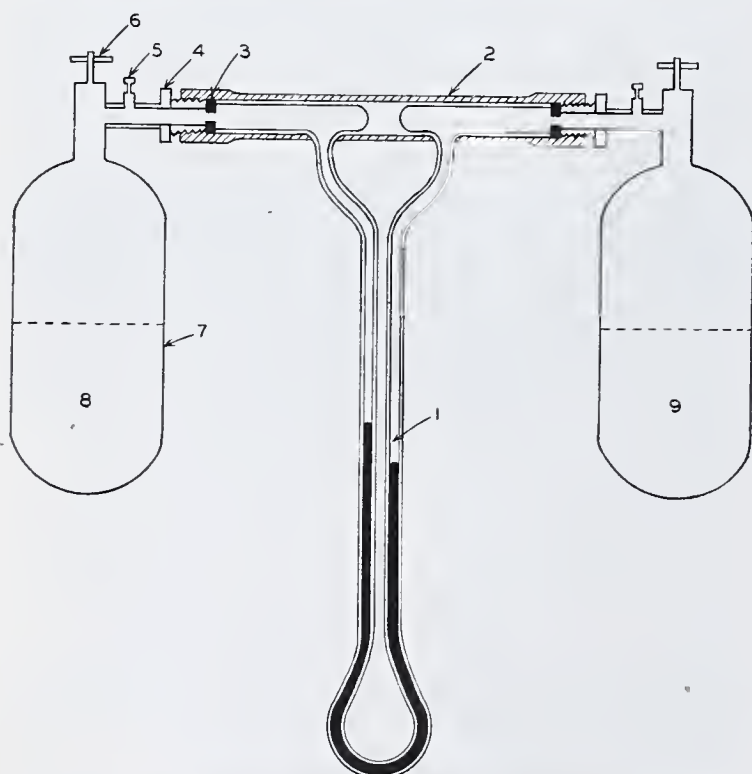


Figure 2. Differential Manometer Apparatus to Determine Vapor Pressure Lowering of Liquefied Gas Solutions

- |   |                              |
|---|------------------------------|
| 1. Heavy-walled glass tube containing mercury                   | 5. Petcock                   |
| 2. Brass frame from 0.5-inch pipe having slots cut in each side | 6. Needle valve on container |
| 3. Rubber gasket  | 7. Container                 |
| 4. Adapter from 0.375- to 0.125-inch pipe thread                | 8. Liquefied gas solution    |
|   | 9. Liquefied gas             |

both sides were used. The exact amount of Freon needed for solution was introduced to avoid fractionation by the removal of any excess. The results are shown in Table I.

Since the total vapor pressure of Freon-12 is about 5000 mm. of mercury, the degree of reproducibility shown is considered good.

Fifteen per cent of nonvolatile material has been found to be about the optimum that should be used in an aerosol solution. Such a solution will have a vapor pressure about 36 mm. of mercury below that of Freon-12. Reference to the equation of state derived for Freon-12 by Buffington and Gilkey (2) shows that this lowering of pressure produces a change in vapor density of only 0.0003 gram per cc., which for the authors' purposes can be considered negligible in comparison with the figure 0.0377 gram per cc. used in constructing the graph.

The possible changes in liquid density were evaluated by consideration of the values for density of solutions of sesame oil in Freon-12, determined at 80° F. by means of a small hydrometer in a closed system.

The liquid was placed in a pressure test tube together with the hydrometer. The whole apparatus (Figure 3) was set in a glass water bath at 80° F. and the length of the emergent stem was determined with a cathetometer. The hydrometer was calibrated by observing the length of the emergent stem above the Freon at several temperatures. The error of this calibration due to the increasing vapor density above the Freon was calculated and found to be negligible. A curve was plotted from which the densities of the various sesame oil solutions were determined at 80° F. (26.7° C.). The values obtained are shown in Table II.

Since 15% of oil is taken as the optimum, the change in liquid density will not exceed 0.048 gram per cc., which would produce a change of only about 0.3 in the value of  $100 \frac{c - C}{C}$  cal-

culated for the case in which 90% of the contents of the test tube is withdrawn. Thus it appears that the graph is sufficiently accurate for all ordinary purposes.

As an objective confirmatory test, measurements of the increase in concentration were made on 400-gram samples of approximately 5% solution of cottonseed oil in Freon-12. Cotton-

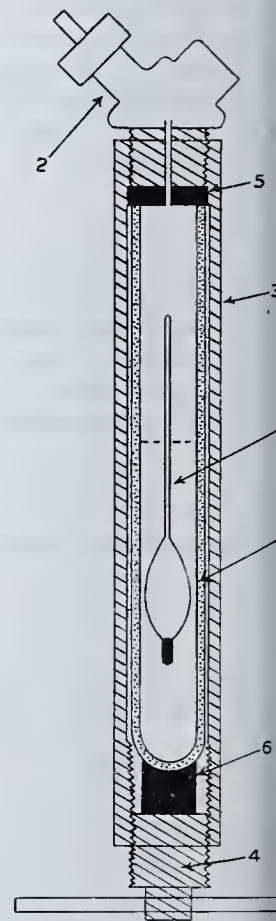


Figure 3. Pressure Test Tube Assembly and Small Hydrometer

1. Heavy-walled glass test tube 10 mm. in inside diameter and 155 mm. long
2. Standard Y-valve for refrigerant drums
3. Frame from 0.5-inch pipe with long windows in opposite sides
4. Screw plug
5. Gasket
6. Rubber cushion
7. Hydrometer

Table II. Change in Density of Freon-12 Due to Sesame

Sesame Oil %	Density G./cc.
0	1.303 <sup>a</sup>
2.5	1.298, 1.2985, av. 1.298
5.0	1.290, 1.292, av. 1.291
10	1.274, 1.274, av. 1.274
15	1.2548, 1.2552, av. 1.255
20	1.2285, 1.2295, av. 1.229

<sup>a</sup> From Bichowsky and Gilkey (1).



### Table III. Concentrating Effect Caused by Removal of Liquid from an Aerosol Bomb

Portion Withdrawn % by weight	Relative Concentration of Oil	
	Determined	Calculated
0	(100.0)	100.0
50	100.9, 101.5, av. 101.2	102.1
80	105.3, 106.5, av. 105.9	105.2
90	108.9, 109.1, av. 109.0	108.0

oil was chosen instead of sesame oil because it did not oxidize when heated for the analyses. The initial concentration was determined by withdrawing two 5-gram samples into pressure tubes, which were weighed before and after to determine the net weight of the samples.

The apparatus was used without the hydrometer. The volatile solvent was then allowed to evaporate, and the test tube containing the residue was removed from the frame and heated for 30 minutes at 110° C. The weight of the residue was then determined and the concentration by weight of nonvolatile matter calculated. Duplicate samples were also taken after 50, 80, and 90% of the solution had been allowed to escape. Mechanical difficulties made the results unreliable after 95% had been removed. All operations were carried out at 80° F. The results are shown in Table III.

The degree of concordance shown is good, considering the experimental difficulties involved. The over-all effect is compara-

tively small until the container is almost empty and, since it is in the direction leading to greater assurance of getting the required minimum concentration, not very important in the actual application of the insecticide. It might be important, however, to a manufacturer filling small containers from a large one. The last containers to be filled will contain more insecticide than the first unless some compensatory measures are taken. It is also important when samples of solution are being used for test purposes as a standard of comparison. In precise laboratory tests it would be good practice to use not more than 50% of the original solution. The simplest procedure in the commercial filling of aerosol containers is to add sufficient pure Freon to the reservoir at intervals to maintain approximately the original concentration. This procedure is used by some present aerosol manufacturers.

Although the foregoing discussion has been based wholly on data pertaining to dichlorodifluoromethane, the formula developed will obviously apply to all liquefied-gas solutions for which the densities of the liquid and gaseous phases remain reasonably constant during evaporation.

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## A Modified Bailey Buret

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THE San Antonio Army Service Forces Depot Laboratory has, for some time, been analyzing large numbers of samples of mayonnaise and other semisolid salad dressings. Tests have been made according to the methods of the Association of Official Agricultural Chemists (1).

Weighing out such samples with the Bailey weighing buret (2) has never proved satisfactory. The only size available to the laboratory has been the 30-ml. capacity which holds an insufficient quantity of salad dressing if duplicate determinations are to be made. Salad dressings, moreover, are of such consistency that there is almost no flow of material from the tip of the buret due to gravity alone. Forcing the sample out with the plunger is a slow process and results in the accumulation of a considerable quantity of material on the adapter and on that portion of the neck which projects above. Such a situation inevitably results in loss.

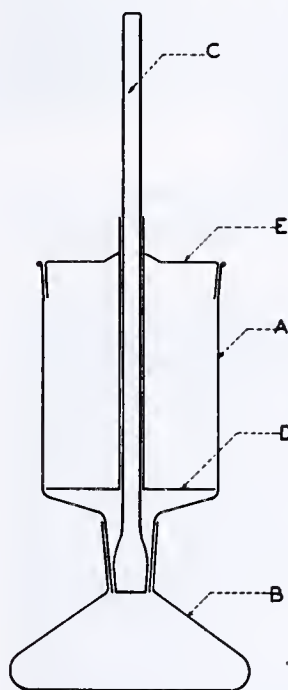
A fairly simple modification of the Bailey buret was decided upon as the best solution of this problem. The changes involved were enlarging the buret to a capacity of 100 ml., straightening the top of the buret completely, eliminating the constriction, and adding a plunger to go down inside the buret and around the neck. All clearances were kept to a minimum.

Buret A is constructed from 51-mm. Pyrex glass tubing with a 50/12 J joint at top and inside 15/20 and outside 15/20 J joint at the constricted bottom. Distance between the joint and beginning of constriction is 50 mm. Flask B has a 15/20 J joint and over-all diameter of 67 mm. Plug C is a 6-mm. glass rod, 165 mm. in length (over-all), and contains inside a 10/18 J joint.

Plunger D is of 18-8 8-mm. (1/32-inch) stainless steel, consisting of a tube and disk spot-welded together. Inside diameter of the tube and diameter of disk are of size to give snug fitting around the neck and inside buret, respectively. Adapter or stopper E is ground and has inside 50/12 joint and orifice to fit over the neck of D.

The modified buret is filled while sitting on the flask base B, with plug C in place. Plunger D is then fitted around the plug

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and allowed to rest on the material. The adapter, E, is finally placed in position, and the assembly is weighed. To remove the sample, the plug is raised and held in open position and, with the same hand, the plunger is pressed downward, forcing out the material. When enough sample has been taken, the plug is pushed into the joint, pressing out the last drop before the buret is returned to the flask.

It is immediately apparent that the total weight of the assembly, filled, is too great for the capacity of an ordinary analytical balance. The assumption is that the larger samples are to be weighed on a more rugged balance, an accuracy of 0.1 or at most 0.01 gram being adequate.

The modified Bailey buret should find use in analyzing samples, such as soft grease, paste paints, certain asphalts, water-repellent emulsions, and other semisolid materials. The plunger, too, can always be removed and the buret can be used to an advantage for any bulky sample.

Satisfactory working models of this buret were obtained from the Scientific Glass Company, Bloomfield, N. J., and have been in continuous use with marked success during the past year. Steps are now being taken to remove minor defects in the design.

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# Determination of Vitamin A Content of Margarine

## Spectrophotometric Method

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A spectrophotometric method for the determination of vitamin A in commercial margarine has been developed. It is based on the destruction of vitamin A in a portion of a solution of the unsaponifiable fraction of margarine fat by ultraviolet light irradiation, and the use of this devitaminized solution as a control for the spectrophotometric determination of vitamin A in a second portion of the original unsaponifiable solution not irradiated with ultraviolet light. The validity of the method is established by a comparison of the results obtained with results of biological assays made on identical samples.

THE need for a rapid and accurate method for the determination of the vitamin A content of margarine has existed as long as margarine has been fortified with vitamin A, but has become more pressing during the last few years as a result of the present governmental nutritional program which encourages the enrichment and fortification of certain foods with essential nutrients. In 1941, a federal definition and standard of identity for oleomargarine (7) was promulgated, which requires that margarine vitaminized with vitamin A must carry not less than 9000 U.S.P. units of vitamin A per pound in the finished margarine. Since this standard has been in effect, almost all margarines on the market carry vitamin A; hence, a dependable and rapid method for determination of vitamin A in finished margarine has recently become relatively more important.

The biological method is too time-consuming for production control purposes. In addition, wide variations in results obtained by the biological method must be expected (2), leaving much to be desired from the standpoint of accuracy. A physical-chemical method is therefore desirable.

### LITERATURE REVIEW

There is relatively little published work on this subject. However, sufficient work has been published to indicate an interest in this matter.

Edisbury (6), in describing vitamin A assay methods with special reference to margarine, pointed out that the ultraviolet absorption method, even when applied to the unsaponifiable fraction of the oil, is unreliable for margarine, because of residual absorption of unsaponifiable constituents other than vitamin A. He suggested a spectrophotometric measurement of the absorption due to the antimony trichloride color reaction, made on the unsaponifiable fraction of margarine fat, as an alternative to the biological assay of margarine. The authors' experience with measuring the intensity of the antimony trichloride color reaction as a means of estimating vitamin A has led them to believe that the intensity of color produced with antimony trichloride varies considerably with slight and oftentimes practically unavoidable variations in details of technique and instruments used. Hence considerable variations in results would be expected among different operators and different laboratories using the antimony trichloride method of vitamin A determination for control purposes. In addition, certain ingredients used in some margarines tend to alter the color produced by the action of antimony trichloride on vitamin A.

Oser (10) indicates that an adaptation of the Dann and Evelyn (3) antimony trichloride method is useful for a quantitative control of vitamin A in margarine. The improvements in this method consist of a correction for side reactions which sometimes develop interfering color or turbidity, and the inclusion of a density measurement produced by the addition of a known increment of vitamin A. The Evelyn photoelectric colorimeter is claimed to increase the accuracy of reading the blue color developed at its maximum intensity. However, this method still depends on being able to read, at its maximum intensity, the unstable and somewhat fleeting color developed by the reaction of antimony trichloride with vitamin A. The instability of the color developed is troublesome for control purposes.

This laboratory (9) has published a spectrophotometric method for determination of vitamin A in dairy butter, based on: the fact that vitamin A is characterized by a maximum absorption at the 3280 Å. band and can be measured by determining the intensity of absorption in the ultraviolet region at this (3280 Å.) wave length, as pointed out by Morton *et al.* (5, 8) and the fact that vitamin A is destroyed by ultraviolet light; with recent years, Demarest (4) has published the results of his study on the destructive irradiation of vitamin A. Thus by destroying the vitamin A (and carotene) in a portion of the unsaponifiable fraction, a control is obtained for the spectrophotometric determination of the vitamin A and carotene contained in the sample. This method is considered to yield satisfactory results.

### EXPERIMENTAL

Attempts were made to apply this spectrophotometric method (9) to margarine, following the procedure used for dairy butter; however, certain modifications are necessary. The difficulties apparently are due primarily to the fact that the unsaponifiable content of domestic U. S. vegetable oils, from which most present-day U. S. margarines are made, differs considerably, both in composition and amount, from the unsaponifiable content of butterfat. Another factor concerned is the fact that butterfat may be more readily saponified than domestic vegetable oils.

Preliminary experimental work indicated that most progress could be made by modifying the method as applied to dairy butter (9) in the following respects:

1. Increasing saponification time in order to ensure complete saponification of the margarine fat before extraction of the unsaponifiable matter.
2. Increasing the number of extractions and the quantity of solvent used for extraction of the unsaponifiable, in order to insure complete extraction of the unsaponifiable material containing vitamin A.
3. Increasing the time of ultraviolet light irradiation of the unsaponifiable in solvent solution, in order to overcome the masking effect of the relatively large amount of unsaponifiable material present in margarine fat and thus to obtain complete destruction of the vitamin A contained therein.

For use in this determination, all solvents must be exceedingly pure—a point which cannot be overemphasized. The solvent for the unsaponifiable material extracted from the oil must be optically clear, must possess adequate solvent power to hold in solution at normal room temperatures the amount of unsaponifiable present in the sample, must be of sufficiently high boiling point to permit long exposures under an ultraviolet lamp which generates considerable heat, and must have no destructive effect upon the vitamin A dissolved therein, at least for several hours.

The authors have found either cyclohexane or methyl cyclohexane, specified as "purified for spectrophotometric use and free of extraneous ultraviolet absorption" and obtained from Eastman Kodak Company, Rochester, N. Y., to be satisfactory in most instances (9). Vitamin A is stable in either of these solvents for several days, provided that the solutions are stored in the dark, and the solvents themselves are sufficiently pure.

It has been the authors' practice to verify the suitability of each lot of either cyclohexane or methyl cyclohexane by spectrophotometric comparison with a sample of known purity. A sample is considered usable only if it shows no extraneous absorption in the region between 5000 and 2200 Å.; extraneous absorption within this range is considered evidence of impurities and the material is rejected. A method satisfactory for the purification of cyclohexane containing a small amount of impurities has been given (9), but is not satisfactory for the purification of methyl cyclohexane.

The ether and alcohol used for extracting the unsaponifiable material must be especially pure and free of peroxides, in order



void oxidation of the extracted vitamin A. These solvents must be carefully purified before use, even though the best grades are purchased. A method for the purification of anhydrous c.p. ethyl ether was given in a previous publication (9). Specially denatured 95% alcohol can be satisfactorily purified by an A.O.A.C. method (1).

Repeated attempts have demonstrated that the vitamin A content of whole margarine fat (vitaminized) cannot successfully be destroyed by ultraviolet light irradiation, using available ultraviolet light equipment. However, the vitamin A content of the unsaponifiable fraction of margarine fat can be destroyed by intense and ultraviolet irradiation. A considerably more transparent solution of the unsaponifiable fraction, in the range below about 3200 Å., results from the intense ultraviolet irradiation required to destroy completely the vitamin A in the unsaponifiable fraction of margarine fat; at 3280 Å., the increased transparency due to the action of ultraviolet light on unsaponifiable materials other than vitamin A is so small as to be neglected for all practical purposes. This point is demonstrated by the results shown in Table I.

The points covered in Table I are further illustrated in Figure 1, in which are shown ultraviolet absorption curves obtained on samples not included in the table. These curves, obtained from the unsaponifiable fractions of the same stock of margarine oil, serve to emphasize:

The absorption due to constituents other than vitamin A in margarine oil unsaponifiable.

Ultraviolet irradiation of the unsaponifiable fraction of a vitaminized oil produces a material which shows no vitamin A characteristics spectrophotometrically. This irradiated material shows absorption, at the point of maximum vitamin A absorption, in reasonably close agreement with that of the nonvitaminized, nonirradiated, unsaponifiable fraction of the same oil.

Irradiation increases the transparency of the unsaponifiable fraction in regions below about 3200 Å.

Irradiation of the unsaponifiable fraction of a vitaminized margarine oil produces a control for spectrophotometric vitamin A determinations, which gives results in reasonably close agreement with those obtained by use of the unsaponifiable fraction of the same oil before vitaminizing.

The curve (curve 5) obtained by the method described herein

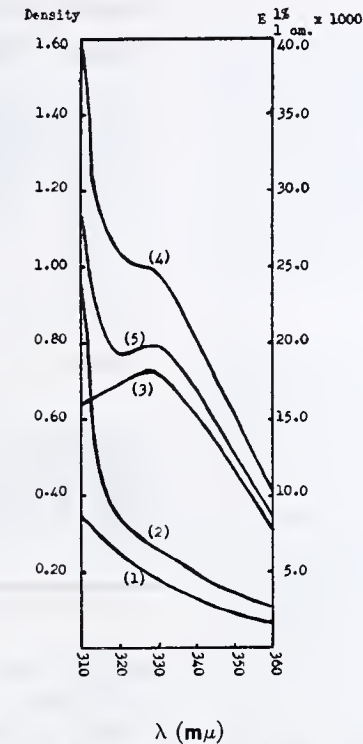


Figure 1. Ultraviolet Absorption Curves

- (1) Irradiated unsaponifiable fraction of vitaminized margarine oil vs. solvent control
- (2) Nonirradiated unsaponifiable fraction of same margarine oil before vitaminizing vs. solvent control
- (3) Nonirradiated unsaponifiable fraction of same vitaminized margarine oil vs. nonirradiated unsaponifiable fraction of same oil before vitaminizing (both in solvent)
- (4) Nonirradiated unsaponifiable fraction of same vitaminized margarine oil vs. solvent control
- (5) Nonirradiated unsaponifiable fraction of same vitaminized margarine oil vs. same material after irradiation (both in solvent)

exhibits a vitamin A peak at the region of maximum vitamin A absorption.

The following method is based on the destruction of vitamin A in a portion of a cyclohexane (or methyl cyclohexane) solution of the unsaponifiable fraction of margarine fat by ultraviolet light and the use of this devitaminized solution as a control for the spectrophotometric determination of vitamin A in a second portion of the original unsaponifiable solution not irradiated with ultraviolet light.

EQUIPMENT USED

**SPECTROPHOTOMETER.** Adam Hilger, Ltd., intermediate quartz spectrograph with Spekker photometer, equipped with tungsten steel electrodes as a source of light. Quartz absorption cells, Hilger Type C, 1-cm. quartz Kjeldahl-shaped flasks, 25 cc.

**Beckman quartz spectrophotometer, Model D,** manufactured by National Technical Laboratories, South Pasadena, Calif. Equipped with tungsten lamp light source, cesium oxide and blue sensitive phototubes, and Corex absorption cells (1-cm. square type).

Both instruments have been used with equally satisfactory results for this determination.

**ULTRAVIOLET LAMP.** Uviarc poultry treater, Type RT, Spec. 100, Cooper-Hewitt Electric Co., Hoboken, N. J. A more complete description of this lamp, together with a reference for obtaining its spectral radiation, has been given (9).

METHOD

**EXTRACTION OF UNSAPONIFIABLE MATERIAL.** Melt the margarine in a water bath at about 60° C., and separate the fat by filtration through a Whatman No. 12 folded filter paper (or other equivalent paper).

Saponify 20 grams of the separated and filtered fat with 30 cc. of alcoholic potassium hydroxide (200 grams per liter of specially denatured No. 30 alcohol) by boiling, with suitable reflux arrangement, for 15 minutes.

Dilute the alcoholic solution with water to approximately 4 volumes and cool in an ice-water bath. Extract the unsaponifiable material with cold ethyl ether. At least six extractions, with the following successive amounts of ether, are required for complete removal of the unsaponifiable material: 200, 150, 100, 50, 50, 50 cc. (Both the ether and alcohol must be very carefully purified immediately before use. This is essential for dependable results.) Vigorous shaking is also necessary for complete removal of the unsaponifiable material. The sample should be adequately protected from sunlight during extraction.

Composite the ether extracts and wash with 150-cc. portions of distilled water until substantially free of soap. (There should be no appreciable turbidity developed after acidifying the wash water with 10% hydrochloric acid.) Adequate precautions should be taken to avoid troublesome emulsions; the first two water washes should be made by merely pouring the water through the ether without shaking.

Filter the ether solution through filter paper and concentrate to 25 to 50 cc. by distillation on a steam bath. Remove the remainder of the ether by evaporating, on a steam bath, under a stream of carbon dioxide to prevent oxidation. When substantially all the ether has been evaporated, cool immediately to about 20° C. (70° F.) and dissolve the unsaponifiable material in optically clear cyclohexane or methyl cyclohexane. Make a solution up to 50 cc. (40% solution weight to volume, on original fat basis). Filter and store in the dark at about 4° to 10° C. (40° to 50° F.) until the sample is examined spectrophotometrically, but not longer than 48 hours.

**SPECTROPHOTOMETRIC DETERMINATION OF VITAMIN A.** Divide the cyclohexane (or methyl cyclohexane) solution of unsaponifiable material into two parts, and irradiate one portion under the Uviarc as follows: Transfer the solution to be irradiated into a 25-cc. Kjeldahl-shaped quartz flask, and stopper the flask with a cork wrapped in aluminum foil. Allow at least 10

Table I. Effect of Irradiation

Comparison of controls used in the unsaponifiable method for determination of vitamin A in margarine oil. Duplicate determinations)

Sample No.	Date Analyzed	Irradiated Unsaponifiable Control	Nonirradiated Unsaponifiable of Oil before Vitaminizing as Control	Irradiated Unsaponifiable of Vitaminized Margarine Oil vs. Nonirradiated Unsaponifiable of Same Oil before Vitaminizing	U.S.P. units of vitamin A per pound of margarine
1	6-11-42	13,600	14,000	600	
		13,600	14,000	600	
2	6-17-42	15,000	14,200	600	
		16,100	15,100	600	

Samples prepared under authors' supervision, with process samples available for use in this experimental work.



minutes for the lamp to come to full operating temperature before starting irradiations.

Support the flask in such a position that the cork rests against the rim of the lampshade (the lamp in use is equipped with a shade 21 cm., 14 inches, in diameter) and the bulb of the flask is held about 10 cm. (4 inches) away from the mercury tube of the lamp. Place a sheet of aluminum foil about 7.5 cm. (3 inches) below the flask being irradiated, in order to reflect the light back toward the sample. Agitate the sample every 15 minutes by gently tapping the flask, for example, with a pencil. Allow the sample to heat up as much as the lamp will heat it, provided that the temperature does not reach the boiling point of the solvent being used.

Irradiate until the vitamin A has been destroyed. Under the authors' conditions, approximately 2.5 hours have been required for complete destruction of the vitamin A contained in this concentration of margarine fat unsaponifiable. Destruction of vitamin A can be estimated by the Carr-Price test, and the time of irradiation required for the ultraviolet lamp in use can be established spectrophotometrically by irradiating until there is no further decrease in absorption of the irradiated sample at 3280 Å.

After cooling to about 21° C. (70° F.), filter the irradiated solution, which must be clear and colorless, and determine vitamin A in the nonirradiated solution by means of the spectrophotometer (1-cm. cells), using the ultraviolet irradiated solution as a control. With the Hilger spectrophotometer, expose the plates at density settings ranging from 0 to 1.50 in increments of 0.05, with the exposure time graduated up to about 2 seconds on Eastman No. 33 plates. These plates are satisfactorily developed with Eastman D72, diluted 1 to 2. Considerable time can be saved, without any sacrifice in accuracy, by using a Beckman spectrophotometer. For this instrument, 1-cm. square type Corex absorption cells are satisfactory. A tungsten lamp instead of a hydrogen discharge tube can be satisfactorily used as a source of ultraviolet light.

Read the match point (or density) at 3280 Å., and calculate the  $E_{1\text{ cm.}}^{1\%}$  value of the sample under test. The difference in absorption at 3280 Å., between the nonirradiated and irradiated sample, is a measure of the vitamin A content of the sample.

This method has been successfully used for determining the vitamin A content of a variety of domestic vegetable oil margarines, but no attempts have been made to apply the method to animal fat margarines or to coconut oil type margarines.

#### CALCULATIONS

$$\frac{\text{Match point (or density) at 3280 Å.}}{40} \times 2140 \times 454 =$$

U.S.P. units of vitamin A per pound of margarine fat

$$\frac{\% \text{ fat in sample} \times \text{U.S.P. units of vitamin A per pound of butterfat}}{100} =$$

U.S.P. units of vitamin A per pound of margarine

These calculations are based on 2140 as the conversion factor for vitamin A.

**DERIVATION OF CONVERSION FACTOR OF 2140.** The conversion factor to be used for converting from  $E_{1\text{ cm.}}^{1\%}$  value at 3280 Å., to U.S.P. units of vitamin A per gram of oil, was determined for the instruments employed by use of the U.S.P. standard of reference cod liver oil. The method, consisting of  $E_{1\text{ cm.}}^{1\%}$  value determinations, made by the instrument being calibrated, on the unsaponifiable fraction of U.S.P. standard of reference cod liver oil containing 3000 U.S.P. units per gram of oil, has been described (9). The saponification and extraction procedure used for removal of the unsaponifiable material from the reference cod liver oil, for spectrophotometric study, is a modification of the procedure published by Wilkie (12).

Table II shows the results of conversion factor determinations made with the Hilger spectrophotometer, using the current U.S.P. standard of reference cod liver oil containing 1700 U.S.P. vitamin A units per gram of oil. These determinations include results obtained by the use of both cyclohexane and methyl cyclohexane as solvents. In order to minimize the effect of any possible instability of the U.S.P. reference oil, fresh or practically fresh

Table II. Establishment of Conversion Factor (Hilger Spectrophotometer)

Date of Determination	Per Cent Solution (Original Oil Basis)	Solvent	Match Point at 3280 Å.	$E_{1\text{ cm.}}^{1\%}$ Value	Conversion Factor
2-28-41	1.000	Cyclohexane	0.795	0.795	2138
2-28-41	1.000	Methyl cyclohexane	0.795	0.795	2138
6-12-42	1.000	Methyl cyclohexane	0.790	0.790	2152
8-4-42	1.000	Methyl cyclohexane	0.790	0.790	2152
8-4-42	1.000	Methyl cyclohexane	0.800	0.800	2125
8-18-42	1.000	Methyl cyclohexane	0.795	0.795	2138
					Av. 2137

Conversion factor of 2140 used. Similar results were obtained by use of Beckman spectrophotometer.

Determinations dated 2-28-41, 6-12-42, and 8-4-42 were made on fresh samples of reference oil, not previously opened. Determination dated 8-18-42 was made on same sample used on 8-4-42 after storage for 2-week interim at 7° C. (45° F.) in the dark and under an atmosphere of carbon dioxide.

samples of the reference oil were used for each determination as indicated.

**EXPECTED ACCURACY OF METHOD.** In order to determine the limit of vitamin A recovery and the degree of reproducibility which might be expected from the use of this method, several lots of margarine were made under controlled conditions. Samples of the oil going into the margarines were taken before the vitamin A-bearing oils were added. The finished margarines were analyzed by the above method and the oils before processing into margarine were analyzed spectrophotometrically as whole oil using the nonvitaminized but otherwise identical oil sample as a control. Typical results (Table III) show that very close to theoretical recovery can be obtained by this method, and that the results are reproducible to a satisfactory degree.

**CORRELATION WITH BIOLOGICAL ASSAYS.** In order to determine the agreement between the spectrophotometric method and the biological U.S.P. method, several samples of margarine were analyzed by the spectrophotometric method at about the time that portions of the identical prints were being assayed for vitamin A by commercial biological laboratories.

Table III. Reproducibility and Recovery of Vitamin A

Sample No.	Determination No.	Irradiated Unsaponifiable Method	Spectrophotometric Analysis of Whole Oil, Using Non-vitaminized Control Oil	Vitamin A Found by Irradiated Unsaponifiable Method
		U.S.P. units	U.S.P. units	%
I	1	15,500	16,100	96
	2	15,100	15,900	95
II	1	16,900	16,900	100
	2	16,700	16,300	102
III	1	15,100	15,500	97
	2	15,700	15,500	101
IV	1	15,300	15,300	100
	2	15,100	15,700	96
				Av. 98.4

All samples used for this comparison were commercial margarines, received in their original containers; included were several different brands of margarine, made by different manufacturers, and also several prints of one single brand of margarine. The data covering the comparison of results obtained by the above method with those obtained by the U.S.P. biological method were built up over a period of approximately one year.

In order to obtain sufficient data to estimate the biological vitamin A potency of these samples with a reasonable degree of accuracy, the commercial biological laboratories employed for this work were instructed, in most instances, to feed the standard of reference cod liver oil and the sample of margarine under test at two different levels. By means of log-dose interpolation curves, plotting weight response *vs.* sample weight fed, the biological



Table IV. Comparison of Results of Spectrophotometric vs. Biological Methods

(Standard of reference oil containing 1700 U.S.P. vitamin A units per gram used)

Sample No.	Brand	Spectrophotometric Method		No. of Levels Fed		Daily Dose		U.S.P. Biological Method		U.S.P. units vitamin A per pound of margarine, average by log-dose interpolation curves <sup>a</sup>
		Determination No.	U.S.P. units per pound of margarine <sup>a</sup>	U.S.P. reference oil	Sample	U.S.P. reference oil	Sample	Average Gain Weight	On sample	
						Mg.	Mg.	Grams	Grams	
1	A	1	9,700	2	2	0.88	56.7	28.1	23.1	10,100
		2	9,500			1.47	94.5	36.1	35.0	
		3	9,300							
		Av.	9,500							
2	B	1	16,100	2	2	0.88	34.0	30.3	14.0	6,900
		2	16,100			1.47	56.7	39.6	20.9	
		3	15,900							
		Av.	16,000							
3	C	1	15,300	2	2	0.882	53.4	19.9	28.9	17,500
		2	15,100			1.176	69.8	29.0	40.0	
		3	14,600							
		Av.	15,000							
4	D	1	9,700	2	2	0.882	82.5	19.9	38.9	15,300
		2	8,900			1.176	113.5	29.0	51.3	
		3	9,100							
		Av.	9,200							
5	E	1	16,300	1	1	1.176	100.9	37.0	51.9	Over 9,000
		2	16,100			..	..	..	..	
		3	16,200							
		Av.	16,200							
6	E	1	11,500	2	2	0.88	45.4	29.9	27.9	13,500
		2	11,300			1.47	75.6	44.2	40.3	
		3	11,400							
		Av.	11,400							
7	E	1	14,000	2	2	0.88	45.4	28.5	34.3	14,200
		2	12,800			1.47	75.7	51.1	38.4	
		3	13,400							
		Av.	13,400							
8	E	1	16,100	2	1	0.882	..	19.9	..	21,400
		2	15,900			1.176	100.9	29.0	57.0	
		3	16,000							
		Av.	16,000							
9	E	1	15,200	2	2	0.88	45.4	30.3	28.3	12,300
		2	14,800			1.47	75.6	48.2	35.0	
		3	14,000			0.88	45.4	40.3	37.4	
		Av.	14,000			1.47	75.6	57.3	43.3	
10	E	1	12,500	2	2	0.88	45.4	31.4	31.8	11,800
		2	13,400			1.47	75.6	38.6	41.9	
		3	14,000							
		Av.	13,800							
12	E	1	14,200	2	2	1.176	100.9	35.5	47.3	Over 9,000
		2	13,400			0.88	45.4	30.2	37.8	
		3	14,000			1.47	75.6	43.8	47.8	
		Av.	13,800			0.88	45.4	28.9	32.8	
13	E	1	14,200	2	2	1.176	100.9	35.5	47.3	18,700
		2	13,400			0.88	45.4	30.2	37.8	
		3	14,000			1.47	75.6	43.8	47.8	
		Av.	13,800			0.88	45.4	28.9	32.8	
14	E	1	16,500	2	2	1.47	75.6	44.3	55.1	18,300
		2	15,900			0.88	45.4	30.2	37.8	
		3	14,000			1.47	75.6	43.8	47.8	
		Av.	13,800			0.88	45.4	28.9	32.8	
v. (excluding Nos. 5 and 12, on which only single level bio-tests are available)			13,491	..	..	..	..	..	..	14,766

<sup>a</sup> Results rounded off to nearest 100 units.

vitamin A potency was estimated as the average of the response from the levels fed. All biological tests were made by the U.S.P. method. Two commercial biological laboratories, Food Research Laboratories, Long Island City, N. Y., and the Laboratory of Vitamin Technology, Chicago, Ill., carried out the biological tests. However, each sample was bio-tested by only one of the biological laboratories.

The comparative results, shown in Table IV, demonstrated that consistent and reproducible results are obtained by the spectrophotometric method; these results are in as reasonable agreement with the U.S.P. biological method as could be expected when the known variation in results obtained by the biological method is considered (11). It is significant that while in this comparison the biological method gave somewhat higher results than the spectrophotometric method on some samples, the reverse was true on other samples, and the average of all the samples analyzed by the spectrophotometric method is in very close agreement with the average of the same samples tested by the biological method.

A similar condition has been found to exist when comparing biological results obtained by different biological laboratories on identical samples of vitamin A-bearing oils. A separate publication (11) covers several years' experience with vitamin A-bearing oils used for enriching margarine and reports biological results obtained by two different laboratories on each of eleven large lots.

The average of the results on all samples obtained by the two laboratories agree to within 3% although results on individual samples show variations ranging from 2 to 95%.

Thus some individual variations in results obtained by spectrophotometric and biological methods, as shown in Table IV, must be expected because of the relatively poor reproducibility of the biological method. The results on most individual samples compared by the two methods show reasonably good agreement in vitamin A potency. These results are considered to confirm the validity of the spectrophotometric method described above.

#### SUMMARY

A spectrophotometric method for the determination of vitamin A in margarine is based on the destruction of vitamin A in a portion of a solution of the unsaponifiable fraction of margarine fat by ultraviolet irradiation, and the use of this devitaminized solution as a control for the spectrophotometric determination of vitamin A in a second portion of the original unsaponifiable solution not irradiated with ultraviolet light. This method gives consistent and reproducible results, and practically complete recovery of the vitamin A contained in margarine. Two satisfactory solvents and two satisfactory instruments for this determination are described. The validity of the method has been demonstrated by comparison with the U.S.P. biological method on identical samples, by feeding both the sample under test and the



U.S.P. standard of reference oil at multiple levels and estimating the biological vitamin A potency from log-dose interpolation curves.

#### ACKNOWLEDGMENT

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## Qualitative Differentiation of the Methylcarbinols and Methyl Ketones

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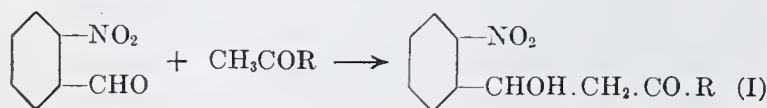
Compounds yielding positive Lieben iodoform reactions may be either methyl ketones or methylcarbinols. On reaction with a reagent containing *o*-nitrobenzaldehyde in alkaline solution, only the methyl ketones will form indigo and may thus be distinguished readily from the methylcarbinols.

IN THE course of a recent investigation, a simple and rapid method was needed for the qualitative differentiation of methyl ketones and methylcarbinols. The valuable Lieben iodoform reaction (7) has long been used to detect the grouping  $\text{CH}_3\text{CO}-$  when joined to a hydrogen atom, or to a carbon atom which does not carry highly activated hydrogen atoms, or groups capable of exerting excessively high steric hindrance. However, the corresponding methylcarbinols ( $\text{CH}_3\text{CHOH}-$ ) will also give the Lieben reaction, owing undoubtedly to their prior oxidation by the alkaline hypiodite reagent to the methyl ketones. Thus, while methyl ketones and methylcarbinols are readily identifiable by the Lieben reaction, there is no simple means of differentiating the two groups.

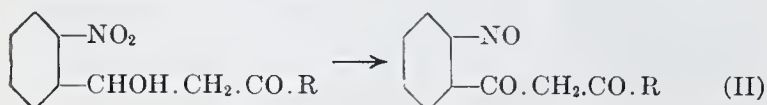
The von Baeyer-Drewsen synthesis of indigo (1, 6), at one time practiced on a commercial scale, is based on a reaction which lends itself readily to such a differential identification technique. *o*-Nitrobenzaldehyde was found by these workers to condense with acetone, acetaldehyde, or pyruvic acid in the presence of alkali, to form indigotin. Feigl, Zappert, and Vasquez (3) applied this reaction to six additional methyl ketones (methyl ethyl ketone, methyl heptenone, acetophenone, acetylacetone, diacetyl, and ethyl acetoacetate) and suggested its applicability in detecting other methyl ketones.

Tananescu and his co-workers (8, 9) have suggested that the reaction probably proceeds as follows:

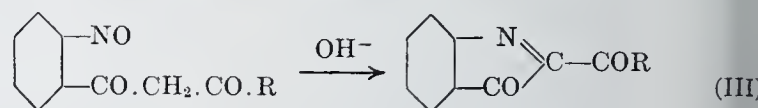
The methyl ketone first condenses with the *o*-nitrobenzaldehyde to form the corresponding  $\beta$ -2-nitrophenyl- $\beta$ -hydroxyethyl ketone (I):



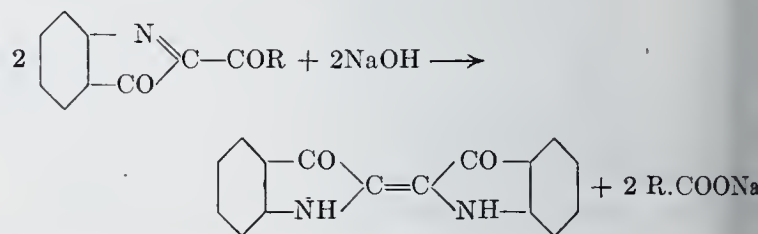
Compound I undergoes an intramolecular oxidation-reduction to form the corresponding  $\beta$ -2-nitrosophenyl- $\beta$ -ketoethyl ketone (II):



Compound II then cyclizes in the presence of the alkali:



to form Compound III, two moles of which split off  $\text{R} \cdot \text{COOH}$  by hydrolysis and form one mole of indigotin:



As the result of the author's investigation, it was found that a simple qualitative differentiation of methylcarbinols and methyl ketones could be based on the Lieben iodoform reaction in conjunction with the von Baeyer-Drewsen indigo reaction, both being specifically modified for use as an analytical technique. A negative iodoform reaction rules out both methylcarbinols and methyl ketones; a positive iodoform reaction and a negative indigo reaction indicate a methylcarbinol; a positive iodoform reaction and a positive indigo reaction indicate a methyl ketone. This differentiation is specific. When a technical sample of a methylcarbinol was found to give a faint positive indigo reaction, this could invariably be traced to the presence of methyl ketone as an impurity. Scrupulous purification of the methylcarbinol (e.g., via a characteristic crystalline derivative) resulted in a product which reacted entirely in the expected manner.

#### IODOFORM REACTION

The procedure used for the iodoform reaction was that described by Fuson and Tullock (5).

REAGENTS REQUIRED. Dioxane, 10% sodium hydroxide solution, and iodine reagent: 200 grams of potassium iodide and 100 grams of iodine dissolved in 800 cc. of distilled water.

PROCEDURE. About 100 mg. of the compound being tested are placed in a 150 × 16 mm. test tube, 5 cc. of dioxane are added, and the sample is dissolved with shaking. First 1 cc. of 10% sodium hydroxide solution and then the iodine reagent



re added dropwise with shaking until a slight excess of iodine causes a definite dark color which does not disappear on standing. The test tube is now placed in a water bath maintained at 40° C., and the dropwise addition of the iodine reagent is continued until the definite dark color persists as before; but the warming at 60° C. should not last over 2 minutes. The excess of iodine is now removed with a few drops of 10% sodium hydroxide solution, and the test tube is filled with cold water, allowed to stand for 15 minutes, and filtered. The characteristic odor of iodoform is readily distinguishable. As a confirmation, the crystals which are collected on the filter paper are dried at 100° C. for one hour and identified by their melting point. Iodoform melts at 119–121° C.

#### INDIGO REACTION

Since *o*-nitrobenzaldehyde is not at present obtainable from any domestic source, it may be prepared by nitrating benzaldehyde by the method described by Friedlander and Henriques (4) and separating the *o*-nitrobenzaldehyde from the mixture of isomers thus obtained by the method described by Ehrhart (2).

**PREPARATION OF REAGENT.** One hundred grams of finely powdered sodium nitrate are added in small portions to 1 liter of 3° Bé. sulfuric acid, the temperature of the mixture being kept below 20° C. by external cooling. C.P. benzaldehyde (106 grams) is now added to this nitrating mixture in small portions, the temperature being kept below 30–35° C. After all the benzaldehyde has been added, the reaction mixture is cautiously poured into a mixture of 1.5 liters of water and 1.5 kg. of ice. The oily layer of mixed *o*- and *m*-nitrobenzaldehyde is separated by decantation and mixed without heating with 500 cc. of 10% sodium bisulfite solution (sp. gr. 1.37). Six hundred cubic centimeters of water heated to 45° C. are now added to dissolve the magma of crystals and the resultant solution is filtered in a refrigerator at 0° to 5° C. for 48 hours. The copious precipitate of *m*-nitrobenzaldehyde sodium bisulfite which forms is filtered off. To the filtrate is added a saturated sodium carbonate solution to strong alkaline reaction, and the mixture is cooled and extracted with three successive 500-cc. portions of ether. The ether extracts are dried overnight over anhydrous calcium chloride, filtered, and the solvent evaporated off. The residual oil (20 to 25 grams) solidifies on cooling and consists of *o*-nitrobenzaldehyde mixed with smaller amounts of *m*-nitrobenzaldehyde. This product is sufficiently pure for use in the technique described below.

The *o*-nitrobenzaldehyde reagent is prepared by dissolving 20 grams of the crystals or the oily product in 100 cc. of 95% ethanol. This reagent solution should be prepared fresh from the undissolved compound at least once a month, and stored in an amber-colored glass-stoppered bottle.

**PROCEDURE.** About 100 mg. of the compound being tested is dissolved or suspended with vigorous agitation in 5.0 cc. of the *o*-nitrobenzaldehyde reagent, and 1.0 cc. of 10% sodium hydroxide solution is added dropwise. An immediate darkening of the solution will occur (partly due to the formation of condensation products of *o*-nitrobenzaldehyde). After 60 seconds, a few drops of the solution are placed on a piece of filter paper and allowed to be absorbed. The filter paper is then washed under a stream of tap water and examined. If the brown stain has washed away, the test is negative—i.e., the sample is a methylcarbinol. A positive test—i.e., a methyl ketone—is evidenced by a distinct and unmistakable deposition of indigo-dyestuff within the fibers of the filter paper. The "spot" of indigo blue is usually rimmed by a characteristic blue-green ring, which cannot be removed, even by prolonged washing. By comparing the washable brown stains obtained with known methylcarbinols and the permanent blue dye obtained with known methyl ketones, the chemist can soon become highly proficient in distinguishing the two.

This method will detect as little as 1 mg. of methyl ketone in a 100-mg. sample of methylcarbinol. It has been tried with a number of methylcarbinols and methyl ketones. The results obtained may be summarized by classifying the compounds as follows:

**CLASS I.** Compounds which give a positive indigo reaction but do not give a positive iodoform reaction: No compounds were found which fall in this class categorically. Sterically hindered compounds like pinacolone (1) give the indigo reaction more readily than they form iodoform, but these belong properly in Class III.

**CLASS II.** Compounds which give a negative indigo reaction and a positive iodoform reaction: ethanol (2), isopropanol (3), methylethylcarbinol (4), methyl-*n*-propylcarbinol (5), methylisopropylcarbinol (6), methyl-*n*-butylcarbinol (7), methylisobutylcarbinol (8), methyl-*n*-amylcarbinol (9), methylisooamylcarbinol (10), methyl-*n*-hexylcarbinol (11), methylisohexylcarbinol (12), butandiol - 2,3 (13), benzylmethylcarbinol (14), 1-phenylpropanediol-2,3 (15), lactic acid (16), methyl lactate (17), and ethyl lactate (18).

This class, therefore, comprises only the methylcarbinols—i.e., the series of compounds characterized by the grouping  $\text{CH}_2\text{CHOH}$ —joined to a hydrogen atom or to a carbon atom which does not carry groups that exert an excessively great steric hindrance.

**CLASS III.** Compounds which give a positive indigo reaction and a positive iodoform reaction: acetaldehyde (19), acetone (20), methyl ethyl ketone (21), methyl *n*-propyl ketone (22), methyl isopropyl ketone (23), methyl *n*-butyl ketone (24), methyl isobutyl ketone (25), methyl *n*-amyl ketone (26), methyl isoamyl ketone (27), methyl *n*-hexyl ketone (28), methyl isohexyl ketone (29), acetoin (30), diacetyl (31), phenylacetone (32), 1-phenylpropanol-1, one-2 (33), pyruvic acid (34), methyl pyruvate (35), ethyl pyruvate (36), methyl cyclohexylketone (37), benzyl acetone (38), acetophenone (39), methyl *p*-tolyl ketone (40), *p*-chloroacetophenone (41), *p*-bromoacetophenone (42), methyl *p*-anisyl ketone (43), 2,4-dimethoxyacetophenone (44), *o*-hydroxyacetophenone (45), *m*-hydroxyacetophenone (46), *p*-hydroxyacetophenone (47), 3-methoxy-4-hydroxyacetophenone (48), *o*-nitroacetophenone (49), *o*-aminoacetophenone (50), 2-aceto-1-naphthoxyacetic acid (51), mesityl oxide (52), benzalacetone (53), salicylalacetone (54), vanillalacetone (55), *p*-hydroxybenzalacetone (56), furfuralacetone (57), acetylacetone (58), acetonylacetone (59), benzoylacetone (60), levulinic acid (61), methyl levulinate (62), ethyl levulinate (63), ethyl acetoacetate (64),  $\alpha$ -acetyl- $\gamma$ -butyrolactone (65), pentanol-1-one-4 (66), 5-diethylaminopentanone-2 (67), methylheptenone (68),  $\beta$ -ionone (69),  $\alpha$ -ionone (70), methyl vinyl ketone (71).

This class, therefore, comprises only the methyl ketones—i.e., the series of compounds characterized by the grouping  $\text{CH}_3\text{CO}$ —joined to a hydrogen atom or to a carbon atom which does not carry groups that exert an excessively great hindrance.

Interfering compounds in the iodoform reaction are usually (a) primary amines which are oxidized to methyl ketones by the alkaline hypoiodite reagent (such as  $\alpha$ -aminoisobutyric acid,  $\alpha$ -phenylethylamine, 2-aminoheptane, 2-aminoheptane, isopropyl amine, etc.), (b) esters of ethanol and secondary alcohols which are hydrolyzed by the alkaline reagent to compounds of Class II (such as ethyl acetate, ethyl propionate, diethylphthalate, diethyladipate, *sec*-butyl acetate, *sec*-amyl acetate, isopropylacetate, etc.), and (c) oximes which are hydrolyzed to methyl ketones (such as acetoxime, acetophenone oxime, acetone oxime, methyl ethyl ketoxime).

The only compounds which have been found to interfere in the indigo reaction are those which are readily hydrolyzed by the alkaline reagent to compounds of Class III. These are (a) halogen derivatives (such as ethylidene chloride, 2,2-dibromopropane, 2,2-dibromobutane, etc.), (b) acetals (such as acetaldehyde alcoholate, acetal), (c) oximes (such as acetoxime, acetophenone oxime, acetone oxime, methyl ethyl ketoxime, etc.), and (d) bisulfite addition products (such as acetaldehyde sodium bisulfite, acetone sodium bisulfite, etc.).

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# Pigment Determination in Carbon Black and Iron Blue Paints

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A simple, rapid, and accurate method for quantitative separation of very fine pigments from oil and varnish vehicles is based upon the fact that certain materials yield light, flocculent precipitates which settle rapidly, carrying along the pigment that is present.

**A** RELIABLE method for accurately separating very small particle size pigments in oil (3, 5) and varnish (1, 2) vehicles has never been satisfactorily worked out. Carbon black, iron blue, chrome greens containing iron blue, and finely ground whites are the most common causes of trouble. It is sometimes possible to filter the fine pigment through a very fine filtering medium but further trouble is experienced because the filter is clogged by the fine particles. Although in some cases it may not be necessary to make a perfect separation and some of the pigment may be passed through the filter, in other cases an exact determination and a clear filtrate are required. The basic principle of the method described in this article can be applied to any pigment-vehicle combination; it is only necessary to find the proper settling agent to collect the pigment and the proper solvent to dissolve the vehicle and to precipitate the settling agent on the pigment.

Since the pigment particles are too small to be retained on the filtering surface, it is necessary to collect them by adding an agent (nitrocellulose, for example) that when precipitated will carry along with it the finely divided pigment. Two methods are outlined in this article; the principle of each is the same; the methods differ only in application and procedure. The first method, which depends upon nitrocellulose to collect the pigment, can be applied to all pigments; it has the disadvantage that the extracted pigment will contain nitrocellulose, although the vehicle will be free from impurities. This method involves no chemical reaction upon any pigment and can be used on nearly any vehicle except emulsion vehicles or poorly soluble vehicles. Certain vehicles, such as run Congo, short oil alkyds, ester gum, and maleic resins sometimes require special treatment, which consists of redissolving and then reprecipitating the nitrocellulose in order to obtain complete extraction of the vehicle.

The second method makes use of a glyceryl phthalate varnish (the phthalate is added if not already present) as the settling agent, precipitated on the pigment in the form of potassium phthalate. Since alcoholic potassium hydroxide is used in this reaction, no alkali-soluble pigments such as lead chromate pigments can be present. Its principal application is to carbon black and other insoluble pigments, to emulsion vehicles, and to poorly soluble vehicles that cannot be determined by other methods. It is based upon the ordnance method for alkyd resin determination (4) and is very accurate.

## REAGENT

**NITROCELLULOSE SOLUTION.** Add 20 parts by weight of the dry R.S. 0.25-second nitrocellulose to 80 parts by weight of ethyl acetate. After solution is complete, determine the exact nonvolatile content of the solution.

## PROCEDURE

**METHOD 1 (Nitrocellulose Method).** Weigh accurately a 2- or 3-gram sample of the paint or enamel and pour into a tared 50-cc. centrifuge tube. Add 10 cc. of ethyl acetate (85% ester), and stir until sample and ester are completely mixed. Pour into this mixture a weighed sample (about 3 grams) of the nitrocellulose solution, and stir until a smooth mixture is obtained. Precipitate this mixture by adding slowly, drop by drop, about 30 cc. of high-solvency aromatic Hi-Flash naphtha, stirring rapidly during the addition. Then place the tube in a water bath and raise to a temperature of 180° F.; hold at this temperature overnight or until the ester has completely evaporated.

At the end of this time, remove from the water bath, cool to room temperature, refill to top with more high-flash naphtha and centrifuge until the top liquid is clear. Pour off the clear liquid and refill with benzene, place in a water bath at 140° F. and allow to stand for about 1 hour. Cool and centrifuge as before. Repeat the centrifuging with the benzene as before and finally wash with petroleum ether, omitting the water bath. Dry at 150° F. to constant weight. Calculate pigment by subtracting the known amount of nitrocellulose from the total weight.

**METHOD 2 (Phthalate Method).** Weigh accurately 7 or 8 grams of the sample into a 250-cc. stoppered Erlenmeyer flask and add 5 cc. of butyl Cellosolve (ethylene glycol monobutyl ether) to aid compatibility of the vehicle. Shake the flask carefully, so that the sample and Cellosolve are thoroughly mixed. Add 3 or 4 cc. of a long oil glyceryl phthalate varnish (unless already present) and shake until a smooth mixture is obtained. It is not necessary to weigh the glyceryl phthalate varnish, as it does not enter into the calculation. In case a smooth mixture does not result at this point, add more Cellosolve slowly until a smooth mixture is obtained. Next add 125 cc. of 0.6 N potassium hydroxide in alcohol (5 grams of potassium hydroxide) and shake thoroughly. Stopper and allow to stand about 3 hours at 130° F. to allow for precipitation. At the end of this time remove from oven, cool to room temperature, add 50 cc. of ethyl ether, and restopper; allow to stand at least 1 hour. Filter through a tared dry filter paper (Whatman No. 32, for example), washing with 50 cc. of alcohol-ether (1 to 1 mixture, using five 10-cc. portions. Dry in an oven at 200° F. for 10 or 15 minutes, remove from oven, and replace in filtering funnel. Wash with warm water (160° F.) until all the soluble potassium phthalate is dissolved and the pigment is washed free of the phthalate. Make the final washing with ethyl alcohol to shorten the drying period. Dry in an oven at 220° F. to constant weight.

Table I. Pigment Determinations in Different Vehicles

Pigment	Vehicle	Method	Pigment Theoretical, %	Pigment Determined, %
Carbon black	Congo varnish	Phthalate	6.60	6.60
White pigments	Emulsion	Phthalate	50.0	46.3
Iron oxide	Alkyd	Phthalate	43.7	43.7
Iron blue	Rosin varnish	Nitrocellulose	51.3	51.7
Carbon black	Linseed oil	Nitrocellulose	3.20	3.20
Carbon black	Alkyd varnish	Nitrocellulose	3.0	3.4

Because of the great variety of enamel and paint vehicles many different types of solubilities are encountered. Table I shows some applications of the two methods and the results obtained. Since the samples were all commercial grades and subject to slight variations, the results are reasonably close. The use of nitrocellulose limits the vehicle solubility to esters and aromatic hydrocarbons. However, the number of cellulose derivatives is large enough to cover practically all vehicles. Ethyl cellulose, benzyl cellulose, cellulose acetate, cellulose acetate butyrate, and other agents may be used for vehicles in which nitrocellulose is not satisfactory. The choice of liquids is very extensive. For solvents there are esters, ketones, and ethers; for precipitating liquids there are aromatic and aliphatic hydrocarbons, terpenes, and alcohols. The choice is determined by the ability of the cellulose product to precipitate on the pigment while the vehicle remains in solution.

The precipitation method of pigment separation results in considerable time-saving in most cases. The material filters very rapidly on a vacuum filter and does not clog the filter. Actually only about 0.5 to 0.75 hour is the total time necessary for the complete operation, including weighing, filtering, and washing. There are many variations in the procedure outlined in this article and the proper application of the general principles of the method should yield accurate results in most cases.



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## A Device for Renewing the Filter-Cake Surface in Small-Scale Vacuum Filtrations

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THIS note describes a device which has been used successfully on a laboratory and semipilot-plant scale to expedite the vacuum filtration of alkaline aqueous dispersions of vegetable protein extracted from peanut and cottonseed meals. Its effectiveness suggests that it might prove useful in clarifying various gummy or colloidal solutions. It can be easily and inexpensively constructed.

The device consists of a hand-operated rotary scraper which can be used with either a table-top Büchner funnel or a stoneware suction filter. By means of the scraper fine colloidal and gummy materials which accumulate on and clog the surface of the filter cake can be periodically removed during filtration along with a thin layer of the filter aid used. By means of this periodic scraping the filter cake surface can be renewed as frequently as is necessary to maintain the filtration rate at a maximum.

iron and a threaded bronze block screwed to the top of the frame. Forty micrometer threads per inch are cut on the shaft and in the block. Although a much thinner cake is ordinarily used, it was considered desirable to cut enough threads on the shaft to allow the scraper to travel through about 3 inches of cake. As the clogged filter aid and gummy material are scraped from the surface they collect in a cone-shaped mass (Figure 1, center) which can be removed whenever a sufficiently large amount accumulates.

A similar scraper designed for use with a stoneware suction filter is shown at the right. The lower bearing is threaded and the scraper assembly is fastened to the top of the filter by screw clamps which are welded to a support resting on the filter.

Better results are obtained when no more vacuum is employed than is necessary to maintain a steady flow through the filter. More rapid filtration of alkaline solutions can be obtained when the filter aid is supported by a glass filter cloth instead of a cotton cloth or filter paper. This is probably due to the fact that glass

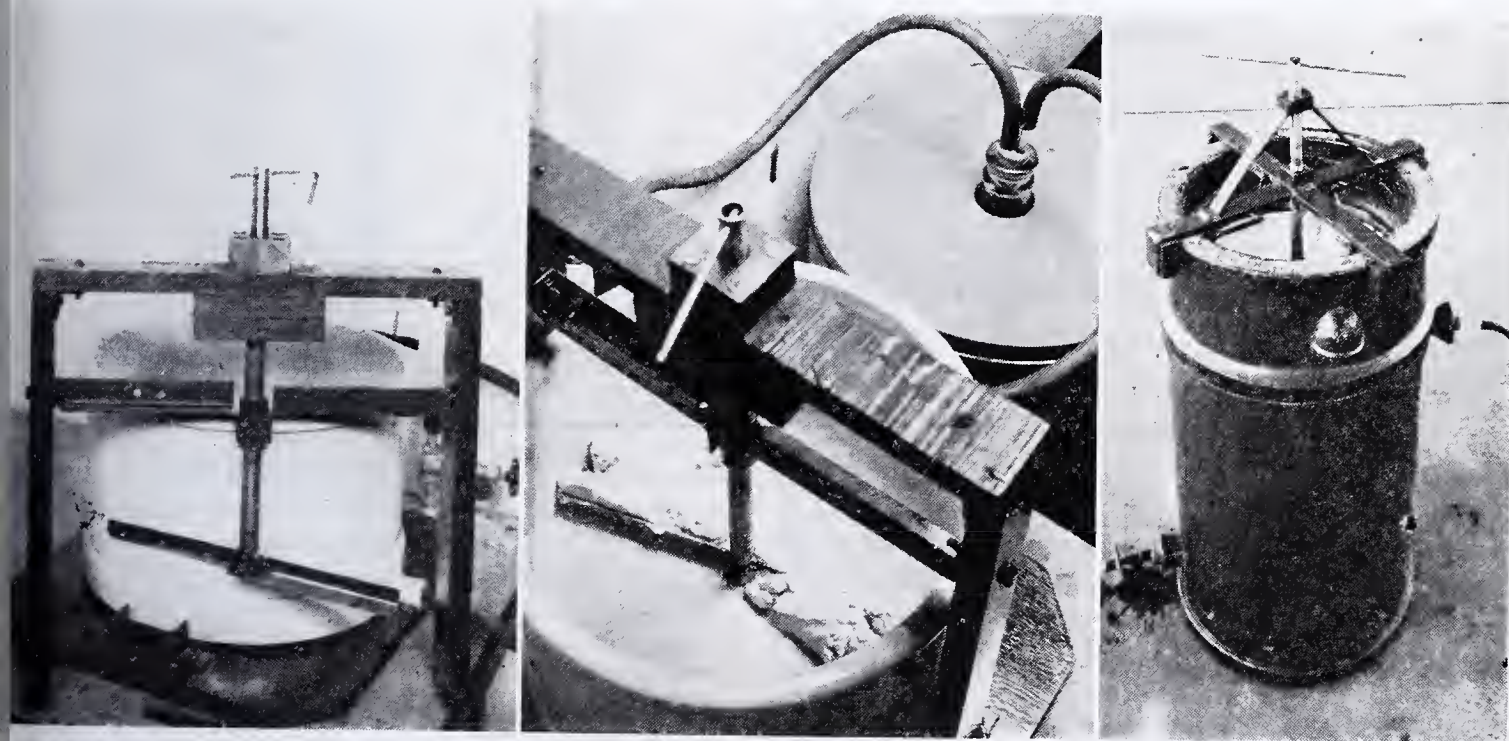


Figure 1. Table-Top Büchner Funnel and Scraper Assembly, Assembled Filtering Unit, and Stoneware Suction Filter with Scraper Attachment

The table-top Büchner funnel, the scraper assembly and plywood base to which it is bolted are illustrated in Figure 1 (left). The assembled unit, including a protected glass bottle for collecting the filtrate is illustrated in Figure 1 (center). The scraper blades are of 1 × 0.125 inch stainless sheet steel bent at an angle of 45° and welded to a shaft of 0.5-inch stainless steel pipe. The outer edges of the blades are sharpened. The shaft is supported by a bearing attached to the wooden frame by two pieces of angle

fibers, unlike cellulose fibers, do not swell and slow the rate of filtration when in contact with alkaline solutions.

The principle of this laboratory filter cake scraper is similar to that used on one type of commercial rotary drum filter manufactured in this country, in that the clogged filter-aid surface is removed by scraping action.



# Determination of Nitrogen Dioxide by Cerate Oxidimetry

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IN THE manufacture of nitric acid by the Ostwald process and its modifications, the amount of nitrogen dioxide in the final acid must be determined. In addition to the effect of nitrogen dioxide in industrial uses of the acid, the amount present is a measure of the efficiency of the air bleach, and represents a waste of the gas. The amount of nitrosyl sulfuric acid in a mixed acid must be determined in order to establish the percentage of the nitric and sulfuric acids present.

The standard method (2) of nitrogen dioxide determination, used in most plants where such work is needed, is titration of a 10-ml. sample with 0.2 *N* potassium permanganate until a pink color persists for 3 minutes. The method is accurate only with careful manipulation of the sample. In actual control practice loss of nitrogen dioxide from the solution under even the best of conditions, speed of addition of potassium permanganate, amount of stirring of the solution while titrating, and individual definition of what constitutes a permanent pink color, as well as the error caused when a rushed analyst shortens the 3-minute period to 2 minutes or even 1—all contribute to inaccuracy. Nitrosyl sulfuric acid is also determined by permanganate titration, using either a 10-ml. sample or an aliquot thereof.

The many published works, and especially those of Smith (1, 3), amply prove the advantages of cerimetry in regard to stability and oxidizing power of the solution, reversibility of the reaction, and lack of side reactions and interferences. These advantages have been retained in developing a method for determination of nitrogen dioxide that is accurate and rapid, even in the hands of an inexperienced analyst.

## REAGENTS

The solutions required are a 0.1 *N* solution of sodium oxalate, an approximately 0.1 *N* solution of ammonium nitrate cerate containing 170 ml. of 72% perchloric acid per liter and the indicator, nitro-*o*-phenanthroline ferrous sulfate. The cerate solution is prepared by adding 55 to 56 grams of ammonium nitrate-cerate to the perchloric acid, stirring for half a minute, and adding 100 ml. of water. The solution is stirred for another half minute, a second 100-ml. portion of water is added, and the process is repeated until a volume of 1 liter is reached. If the salt is not dissolved in this way, an insoluble salt may precipitate in a few days' standing.

The cerate solution is checked periodically in terms of exactly 0.1 *N* sodium oxalate.

## PROCEDURE

To 100 ml. of water in a 250-ml. beaker, 5 ml. of 72% perchloric acid are added. To this acidified solution in most cases 25 to 50 ml. of 0.1 *N* cerate are added, the amount varying with the quantity of nitrogen dioxide expected to be present. A 10-ml. sample of the acid is pipetted accurately into the beaker. At the start of delivery, the pipet tip should be near the bottom of the beaker; near the end of delivery, the tip is raised until it barely touches the surface of the solution. It is let stand 1 or 2 minutes, then stirred slowly. Two to 3 drops of nitroferroin are added, and the excess cerate is titrated with sodium oxalate. The end point is sharp. Since the exact cerate equivalence in terms of a 0.1 *N* solution is known by the earlier oxalate titration of the cerate, the milliliters of cerate actually used in oxidation of the nitrogen dioxide will be the equivalence figure minus the milliliters of 0.1 *N* oxalate used in titration of the residual cerate. The percentage of nitrogen dioxide may be calculated by the usual equations, recognizing that 0.1 *N* solutions have been used in place of 0.2 *N* potassium permanganate.

## EXPERIMENTAL

The method was carefully checked against the potassium permanganate procedure, using a series of acid samples containing varying amounts of nitrogen dioxide. Extreme care was taken

to assure maximum accuracy in the permanganate determination. The potassium permanganate solution was floated upon the surface of the acid water, and not stirred until very close to the end point, when very slow stirring was initiated. The prescribed 3-minute period was taken to assure permanency of the pink potassium permanganate color.

Table I presents data comparing percentages of nitrogen dioxide as determined by the potassium permanganate and cerate procedures.

The determinations by the cerate method were completed in much less time than those by the permanganate procedure. The results compare favorably. In the higher percentages of nitrogen dioxide it is believed that results by the cerate procedure are more accurate than by the permanganate, because of sharper end point and absence of opportunity for possible loss of nitrogen dioxide, since the gas is always in contact with an excess of oxidizing agent.

No attempt was made to extend the method directly to the nitrosyl sulfuric acid determination. Since the potassium permanganate titration for this is identical with that for nitrogen dioxide, the writer can see no reason why the cerate titration should not be entirely satisfactory for this estimation.

Table I. Determination of Nitrogen Dioxide

(In approximately 60% nitric acid)

Sample No.	Nitrogen Dioxide Found	
	Permanganate method	Cerate method
	%	%
1	0.15	0.15
2	0.28	0.28
3	0.36	0.37
4	0.48	0.48
5	0.59	0.60
6	0.80	0.80
7	0.88	0.88
8	1.02	1.02
9	1.24	1.24
10	1.46	1.47
11	1.58	1.59
12	1.69	1.70
13	1.84	1.85
14	2.09	2.10
15	2.26	2.27
16	2.63	2.64
17	2.97	2.98
18	3.49	3.51
19	4.16	4.17
20	5.34	5.36

## SUMMARY AND CONCLUSIONS

A procedure utilizing a cerate solution as the oxidizing agent has been outlined for the determination of nitrogen dioxide, and its application to the estimation of nitrosyl sulfuric acid suggested. The method has none of the disadvantages of the potassium permanganate procedure and may be carried out rapidly. Experimental work has proved it to be fully as accurate as the permanganate method in lower concentrations of nitrogen dioxide and slightly more accurate at higher concentration, under conditions in which extreme care was taken with the permanganate procedure. Under rushed control laboratory conditions, the cerate procedure is much more accurate than the permanganate procedure.

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# Determining Hygroscopicity of Fertilizers

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A rapid method is described for determining the hygroscopicity of fertilizers by measuring the relative humidity of the air in equilibrium with the mixtures. From curves showing these values against the moisture contents, the storage quality of fertilizers can be determined.

THE hygroscopicity of a fertilizer, or its tendency to absorb moisture, determines to a large extent whether it will remain stable under humid conditions and whether it will cake on storage. A rapid and accurate method for measuring the hygroscopicity of a fertilizer would, therefore, be a means of predicting the behavior of the fertilizer under practical conditions.

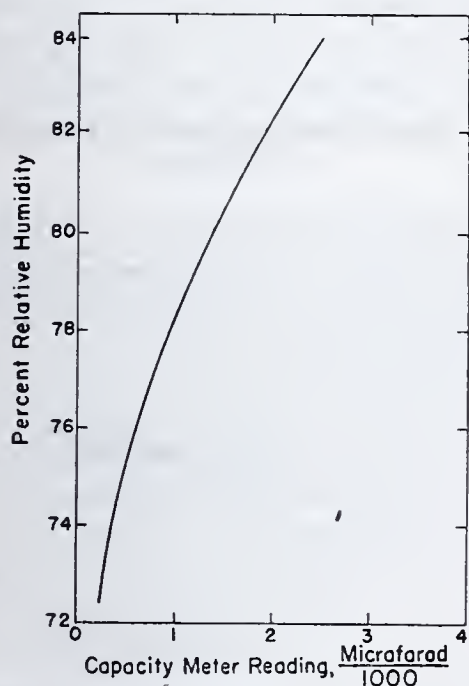


Figure 1. Relative Humidity-Resistance Curve

A fertilizer material will absorb moisture from the surrounding atmosphere if the relative humidity of that atmosphere is greater than that corresponding to the vapor pressure of a saturated solution of the fertilizer material at the same temperature, and will lose moisture at relative humidities below that value. The aqueous vapor pressure of a saturated solution is thus a measure of the hygroscopicity of the solid in question. Adams and Merz have determined the hygroscopicity of a large number of pure fertilizer compounds and their mixtures by measuring the relative humidity over their saturated solutions by means of an isoteniscope. This method, however, requires the evaporation of a large quantity of water from the liquid phase and is, therefore, not applicable to mixed fertilizers which generally contain only a small amount of moisture. The withdrawal of much water would disturb the equilibria in such systems.

Ordinarily, when it is desired to determine at what relative humidity a fertilizer mixture begins to take up moisture, a number of tared samples are exposed to various known relative humidities in controlled-humidity chambers. The relative humidity at which the sample just begins to gain weight is noted. This is the threshold value above which the fertilizer mixture will absorb moisture and below which it will not. This method,

however, will not always give the exact values because it is impractical to prepare humidity chambers to cover the entire humidity range in narrow intervals. The method described in this paper, however, permits accurate determinations of the hygroscopicity of fertilizers in about 30 minutes with no weighings and without the humidity chambers required for the usual method.

Over each fertilizer mixture, there must exist a partial aqueous vapor pressure corresponding to the vapor pressure of the complex solution contained in that particular fertilizer mixture. If, therefore, a fertilizer mixture is kept in a closed container until equilibrium is established at a desired temperature, and a method is found to determine the relative humidity over the sample, a measure of the hygroscopicity of the sample will have been obtained.

For measuring the relative humidity in a small enclosed space, the electric hygrometer as developed by Dunmore (2) of the National Bureau of Standards, was found to be most satisfactory. The electric hygrometer unit consists of a moisture-sensitive film containing lithium chloride on a bifilar coil of palladium wire wound on a thin-walled polystyrene tube. The resistance between the two terminals of the electric hygrometer varies with the relative humidity to which the unit is exposed. The humidity-resistance calibration curve for one such unit is shown in Figure 1. It requires five units with moisture-sensitive films containing various amounts of lithium chloride to cover the whole humidity range.

## APPARATUS

The arrangement of the apparatus used for this determination is very simple, as shown in Figure 2. A is a 16-ounce bottle about  $\frac{1}{3}$  full of a fertilizer mixture, B. C is an electric hygrometer unit. D and D' are terminals of the unit coming through the two holes in the rubber stopper. These terminals are soldered to copper tips F and F' on top of the glass rods, G and G', coming through the same openings in the rubber stopper to hold the unit in place.

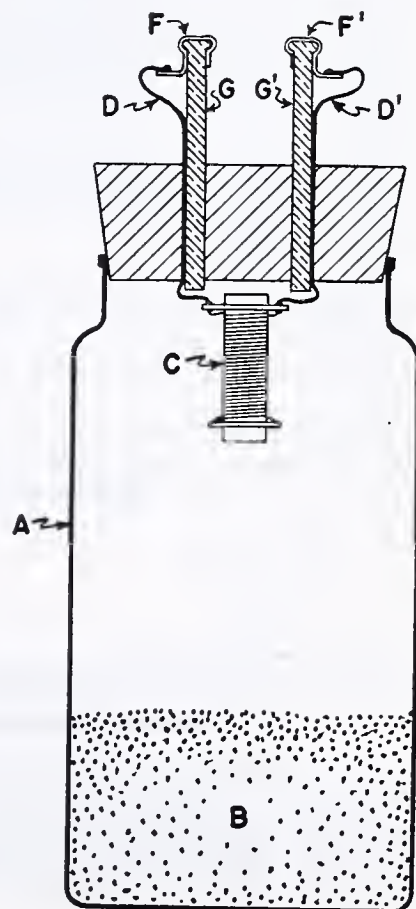


Figure 2. Electric Hygrometer Unit Assembly



Table I. Relative Humidity over Fertilizer Mixtures at 30° C.

Fertilizer Mixture No.	Relative Humidity over Sample by Electric Hygrometer Method	% Moisture Absorbed at Equilibrium at Relative Humidity of		
		59.4%	65.2%	72.5%
1	60.2	-0.27	5.57	14.36
2	59.7	0.01	11.66	23.32
3	59.1	-0.34	7.26	14.32
4	65.5	-1.31	0.40	14.96
5	65.7	-1.59	-0.18	9.70
6	72.6	-1.67	-1.21	-0.55
7	55.6	10.23	17.08	26.07
8	< 51.4	9.04	12.99	22.34
9	64.3	-1.20	2.18	15.65
10	61.9	-0.89	5.12	12.95
11	61.4	-2.44	3.20	11.17
12	67.6	-2.23	-1.38	10.44
13	71.6	-2.31	-1.82	5.43
14	73.2	-1.99	-1.77	-1.28

## PROCEDURE

The procedure for making a determination is also very simple. An electric hygrometer of the proper humidity range is inserted in the bottle containing the fertilizer sample to be tested, as shown in Figure 2, and the whole assembly is allowed to stand for about 20 minutes to come to equilibrium. The resistance between the electric hygrometer terminals is then measured by connecting  $F$  and  $F'$  to a resistance meter (not shown), or a Weston Model 764 capacity meter. The capacity meter may be used as an ohmmeter, since it is measuring only ohmic resistance in a nonreactive circuit (2). A steady reading of the meter signifies that equilibrium has been established. The relative humidity over the fertilizer sample is then read off from the calibration curve for the unit used, like the one shown in Figure 1.

These measurements can be conveniently made in a constant-temperature room, or carried out elsewhere, provided a thermometer is inserted through the rubber stopper to record the temperature of the sample at the time of the measurement. Enough time should be given for the samples to come to equilibrium.

## EXPERIMENTAL RESULTS

In order to test the validity of this method, the relative humidities over a number of well-cured fertilizer mixtures were measured at 30° C. by the electric hygrometer method. The equilibrium moisture absorption values of these fertilizers at 59.4, 65.2, and 72.5% relative humidities at the same temperature had previously been determined. The results obtained for these fertilizer mixtures are tabulated in Table I.

These data show that this method gives consistent results agreeing closely with those obtained by the moisture-absorption method.

Results showing the effect of temperature on the relative humidity over a fertilizer mixture are tabulated in Table II. They reveal that the relative humidity over a fertilizer increases with increase in temperature.

Well-cured fertilizer samples only should be used in making these determinations, because the relative humidity over a raw fertilizer mixture changes as the reactions progress between the various components in the mixture to form more stable salts (3).

Table II. Effect of Temperature on Relative Humidity over a Fertilizer Mixture

Temperature ° C.	Relative Humidity over Fertilizer %
20	62.9
30	68.2
45	73.0

## INTERPRETATION OF RESULTS

By means of a plot showing the relative humidity over a fertilizer against its moisture content, it is possible to judge (a) whether or not the fertilizer contains a large amount of hygroscopic components and whether these components are all in solution or largely in the solid state, and (b) the amount of moisture

the fertilizer will take up at a given relative humidity and at what relative humidity the absorption of moisture will begin with a given moisture content in the fertilizer.

Figure 3 shows three curves, somewhat idealized, that represent relative humidity conditions over three types of fertilizers designated as A, B, and C. An interpretation of these curves will serve to illustrate the points mentioned above.

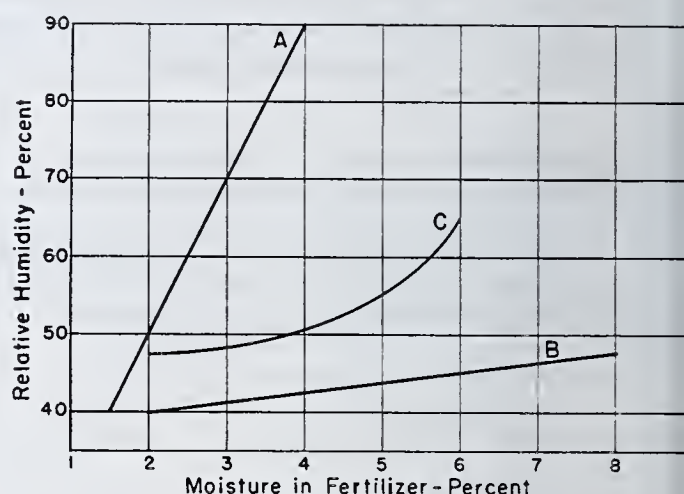


Figure 3. Effect of Moisture Content on Relative Humidity over Fertilizers

The relative humidity over fertilizer A increases rapidly with relatively small increases in the moisture content, indicating that this fertilizer contains only a small amount of hygroscopic components and that these components must all be in solution at a moisture content of only 1.5%. Otherwise the relative humidity over the fertilizer would not increase so rapidly with increase in moisture content, if large amounts of hygroscopic components remained undissolved. This does not, however, exclude the possibility that it contains such salts as potassium sulfate, potassium nitrate, or other relatively nonhygroscopic salts, in the solid state. An O-P-K grade of fertilizer, where the potash is other than manure salts, would exhibit the foregoing properties. Fertilizer A, therefore, is a good mixture because even at 90% relative humidity it will pick up not more than 4% of moisture and not more than 3% at 70% relative humidity. Conversely, curve A reveals that this fertilizer with a moisture content of 4% will not absorb moisture below 90% relative humidity, nor does it begin to absorb moisture below 70% relative humidity when its moisture content is only 3%.

Curve B shows that the relative humidity over fertilizer B increases very slowly as the moisture increases. This means that this fertilizer contains a large amount of very hygroscopic materials and that they are not all in solution at the beginning when the mixture contains 2% of moisture. Fertilizer B, as revealed by the curve, will, therefore, not stand up well under humid conditions because, even when containing 9% of moisture, its relative humidity is still below 45%, and the curve has not even begun to show a break, which means that it still contains hygroscopic substances in the solid state.

Fertilizer C shows little change in relative humidity when its moisture content increases from 2 to 3%, but soon after that curve C starts to turn upward, showing that the more hygroscopic components have all gone into solution and that the solution begins to get more and more dilute with further increase in moisture content. Fertilizer C, intermediate between A and B, is a fairly good mixture because in an atmosphere of 70% relative humidity its moisture content cannot be more than 6%; otherwise the solution present in the fertilizer will have a relative humidity higher than 70%, in which case the fertilizer will absorb more moisture.

## DISCUSSIONS

In actual practice, this type of curves may not always come out as regular as these. It is well known that when a fertilizer once gets wet and then dries again, it becomes a little more hygroscopic than the original mixture. This shows up more with mixtures containing soluble components in large granular forms than those having such components in the fine state. On account of this, the curve obtained by introducing moisture to the mixture may



it quite coincide with the curve obtained by removing moisture from the wet mixture. Once equilibrium has been established, however, the values will become constant.

In routine analysis, a single relative humidity measurement is enough to classify a very wet fertilizer having low relative humidity over it as unsatisfactory, because even with such high moisture content, this fertilizer still contains a concentrated solution of hygroscopic components and some of them may still be in the

solid state. On the other hand, a wet fertilizer having high relative humidity over it may be a good one, provided its moisture content can be reduced to a satisfactory value.

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## Stable Starch Solution for Dissolved Oxygen Determinations

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NUMEROUS starch solutions for use in iodometric titrations including the Winkler (15) determination of dissolved oxygen have been described (1, 4, 8, 9, 12, 14), but the preparation of many of these involves elaborate procedures requiring exact quantities of reagents. Various starch solutions have been criticized (2) because on aging they frequently produce a reddish or violet color with iodine, which prevents sharp end-point readings. The method described here not only

One lot of this starch solution was used by the writer over a 12-month period without deterioration, mold growth, loss of potency, or production of reddish color in iodometric titrations for dissolved oxygen. The indicator properties of several starch solutions were compared photelometrically by titrating to final end-point partitioned samples of water prepared by the Kemmerer (7) method for the iodometric determination of dissolved oxygen, in a Cenco Photometer (Figures 1 and 2).

This method of preparing starch solution lends itself to use in the field, yields a solution which develops and maintains greater depth of color per unit, and has a sharper end point than any of the starches tested.

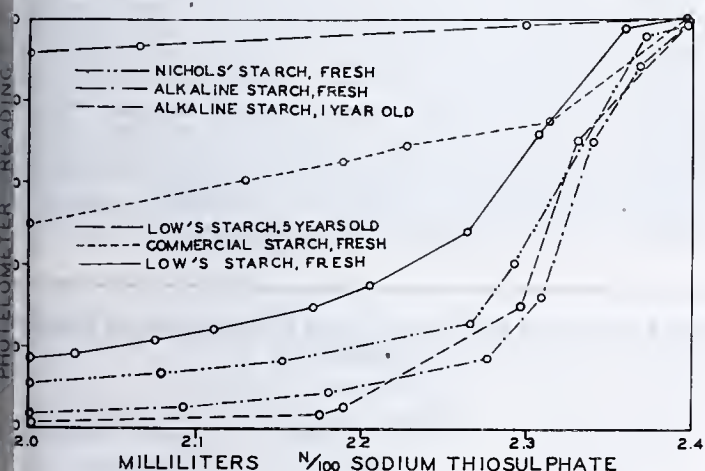


Figure 1. Photelometric Comparisons of Indicator Efficiency of Starches

In iodine in similar samples was reduced by thiosulfate titration in photometer to a reading of 92, exactly 0.2 ml. of test starch added, and titration continued to disappearance of blue starch-iodine color.

eliminates the need for laboratory conveniences and weighed reagents but yields a starch solution which remains unchanged a year or more.

METHOD. Advantage is taken of the property of caustic alkali to dissolve the coating on the starch granules without affecting the starch itself (3, 5, 10, 13).

A 20% solution of sodium or potassium hydroxide, the solid caustic, is added with stirring to a suspension of about 2.0 grams of powdered starch in 10 to 400 ml. of water until a thick, sirupy, almost solid solution is obtained. About 30 ml. of 20% potassium hydroxide are required to treat approximately 2 grams of potato starch. The solution is allowed to stand for about 1 hour to ensure complete action by the alkali, and then made neutral or slightly acid with concentrated hydrochloric acid (6), using litmus paper as indicator. This product is designated "alkaline starch". If acidity does not interfere in the proposed titration (the final sample in dissolved oxygen titration by the Winkler method is acidic), 1.0 ml. of glacial acetic acid is added as a preservative.

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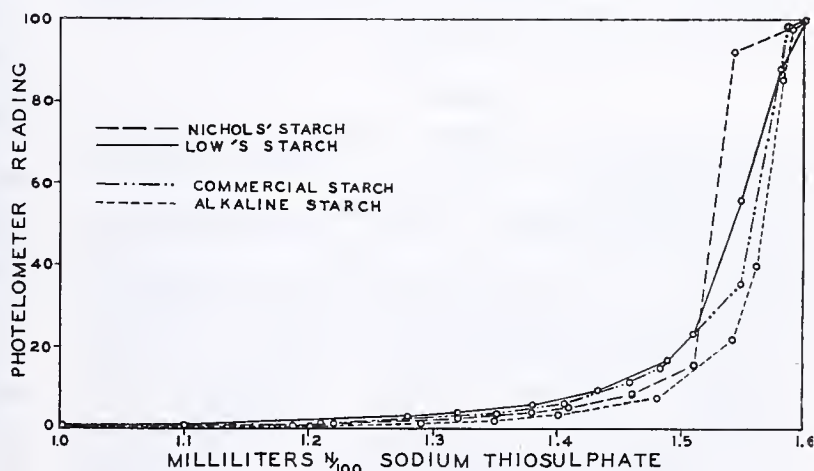


Figure 2. Photelometric Comparisons of Indicator Efficiency of Starches in Quantities Producing Equal Color Intensities

In similar 50-ml. samples free iodine was reduced by thiosulfate titration in photometer to a reading of 82. Sufficient starch solution (1.51 ml. of commercial Lintner's, 0.60 ml. of Low's, 0.41 ml. of Nichols', 0.26 ml. of alkaline) was added to produce photometer reading of exactly 0, and titration continued to clear end point.



# Rapid Estimation of Chlorate Ion Employing Catalysis

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A method for estimating chlorate in cell liquor which is produced during the manufacture of chlorine consists of reducing the chlorate ion in 40% hydrochloric acid by volume with a standard ferrous ammonium sulfate solution. A few drops of a 10% solution of ammonium molybdate are employed as catalyst. The excess standard ferrous ammonium sulfate is then titrated with standard potassium dichromate, using diphenylamine sulfonate as the redox indicator.

SEVERAL methods for the determination of chlorate ion are described in the literature. Bacho (1) reduced the chlorate ion with excess sodium arsenite in hydrochloric acid solution, and then used the potassium bromate method of Györy (4) in determining the excess arsenite. Peters and Deutschländer (6) employed an arsenite-bromide mixture in a strong hydrochloric acid solution. Osmium tetroxide was suggested as a catalyst by Gleu (3). Bølge and Troberg (2) reduced the chlorate ion with excess cuprous chloride at 80° C. and finally titrated the excess with standard potassium dichromate. Harvey (5) used a ferrous sulfate-potassium iodide system, titrating the liberated iodine with standard sodium thiosulfate.

Essentially two methods are employed at Basic Magnesium, Incorporated, for the determination of sodium chlorate in Hooker cell liquor from the chlorine plant. Method II, described in this paper, is a modified Bacho procedure, which differs from the original method in that refluxing of the sodium arsenite-sodium chlorate mixture is omitted; iodine monochloride is employed as a catalyst in the titration of the excess sodium arsenite by potassium bromate, thus sharpening the end point and making the titration possible at lower than boiling temperatures. Smith (7) advocates use of this catalyst in the potentiometric estimation of arsenite by potassium bromate.

A newer procedure for the estimation of chlorate ion, Method I, is being used currently in this laboratory. It consists of reducing the chlorate with an excess of ferrous ammonium sulfate in a strong hydrochloric acid solution, using ammonium molybdate as a catalyst. The excess ferrous ammonium sulfate is titrated with potassium dichromate, using diphenylamine sulfonate as the internal redox indicator.

Method I is preferred because of its high degree of accuracy and precision. It is less sensitive to variable conditions than the sodium arsenite-potassium bromate procedure, in which arsenic is lost if sufficient care is not exercised.

## METHOD I

**REAGENTS.** Ferrous ammonium sulfate, c.p. (0.25 *N*). Potassium dichromate, A.R. (0.1 *N*). Ammonium molybdate, c.p. (10% solution).

**Sodium acetate-phosphoric acid buffer.** Add 250 ml. of concentrated phosphoric acid, c.p., to 1 liter of 4 molar sodium acetate, c.p.

**Diphenylamine sulfonate indicator.** Dissolve 0.30 gram of the barium salt of diphenylamine sulfonic acid in 100 ml. of water, add 0.5 gram of sodium sulfate, and filter off the precipitate of barium sulfate.

**Concentrated hydrochloric acid, c.p.** Hydrochloric acid solution (1 *N*). Phenolphthalein indicator (1% solution).

**PROCEDURE.** Pipet a 10-ml. sample of cell liquor into a 500-ml. Erlenmeyer flask, add 2 drops of phenolphthalein indicator, and titrate with 1 *N* hydrochloric acid. An estimation of total alkalinity sufficiently accurate for cell liquor chemical control may be made at this point. Add 10 ml. of 0.25 *N* ferrous ammonium sulfate solution, 3 drops of ammonium molybdate catalyst, and 40 ml. of concentrated hydrochloric acid. Allow

the mixture to stand 1 minute for complete reaction, then add 20 ml. of phosphoric acid-sodium acetate buffer reagent. Dilute to 200 ml. with distilled water, add 3 drops of diphenylamine sulfonate redox indicator, and titrate with 0.1 *N* potassium dichromate to the purple end point. A correction of 0.05% of dichromate is made for each 6 drops of indicator solution.

## METHOD II

**REAGENTS.** Sodium arsenite, A.R. (0.1 *N* solution). Potassium bromate, c.p. (0.1 *N* solution).

**Iodine monochloride.** Dissolve 0.279 gram of pure potassium iodide and 0.178 gram of pure potassium iodate in 250 ml. of water. Add at one time 250 ml. of concentrated hydrochloric acid (sp. gr. 1.19). The resulting solution is 0.005 *M* in iodine monochloride.

**Concentrated hydrochloric acid, c.p.** Phenolphthalein indicator (1% solution). Methyl orange indicator (0.1% solution).

**PROCEDURE.** Pipet a 10-ml. sample of cell liquor into a 500-ml. Erlenmeyer flask, add 1 or 2 drops of phenolphthalein indicator, and titrate the sample with 1 *N* hydrochloric acid. An estimation of total alkalinity sufficiently accurate for cell liquor chemical control may be made at this point. Add 30 ml. of standard sodium arsenite solution and 20 ml. of concentrated hydrochloric acid and dilute to 100 ml. with distilled water. Cover the flask with a small watch glass and bring the sample to a gentle boil on a hot plate, removing it after 8 to 10 minutes. While the sample is still warm (40° to 70° C.), rinse down the watch glass, add 5 ml. of iodine monochloride and 5 drops of methyl orange indicator, and titrate the excess sodium arsenite with standard potassium bromate. During the titration, the red color of the methyl orange gradually fades to a yellow, until just a few drops before the end point a bright pink color develops. The further addition of potassium bromate will completely destroy the indicator which is considered to be the end point.

Table I. Effect of Catalyst and Acid Concentration on Reduction of Chlorate Ion

Concentration of HCl by Volume	(Reaction time, 1 minute)	
	Chlorate Reduced, Catalyst Present	Chlorate Reduced, Catalyst Absent
%	%	%
18	34.99	32.90
31	91.22	82.45
36	98.40	94.61
40	99.70	98.80
50	99.70	98.80

## DISCUSSION

Table I illustrates the importance of acid concentration in the determination of chlorate ion, using an excess of standard ferrous ammonium sulfate with and without ammonium molybdate as a catalyst. The standard 0.1 *N* solution of sodium chlorate was prepared from Merck reagent quality sodium chlorate crystals; 10 ml. of this solution were employed. The ferrous ammonium sulfate solution was standardized against the standard potassium dichromate solution in both the presence and the absence of the catalyst. The results indicated no interference by the molybdate.

Table II gives a comparison of Methods I and II. The values represent the sodium chlorate content of the samples expressed as milliliters of 0.1 *N* solution. In general, the methods are in close agreement, accounting for about 99% of the chlorate ion present. In routine analyses for control purposes this accuracy is entirely adequate.

Hypochlorite in cell liquor is not considered in this discussion since it is present only in microquantities. The hot alkaline liquor of the Hooker cell promotes the formation of chlorate ion

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## Table II. Comparison of Methods I and II on Hooker Cell Liquor

Sample No.	Method I	Method II
1	2.82, 2.84	2.77, 2.72
2	6.45, 6.41	6.32, 6.22
3	16.77, 16.84	16.77, 16.84

### SUMMARY

After considerable investigation, it is believed that the most accurate method for the determination of sodium chlorate cell liquor produced in chlorine manufacture is the reduction of the chlorate with excess ferrous ammonium sulfate in 40% hydrochloric acid by volume. Ammonium molybdate is used as the catalyst. The method is accurate to within about 1%

of the amount of chlorate present. It permits greater precision than the sodium arsenite-potassium bromate method.

The sample of cell liquor for the sodium chlorate estimation may also serve for a total alkalinity determination if standard hydrochloric acid is used in the initial neutralization process.

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# An Iodine Number Method for Tall Oil

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Use of pyridine sulfate dibromide in conjunction with mercuric acetate catalyst as a bromine addition reagent is suggested for the iodine number determination of tall oil and similar highly unsaturated, conjugated compounds. Data are presented showing the effects of absorption time and excess reagent. Evidence is given that the undesired secondary reaction of substitution does not occur. The iodine numbers of eight different commercial samples of crude tall oil ranged from 237 to 287. This method of iodine number determination has the possibility of general application.

A SATISFACTORY and practical method for determining the iodine number of tall oil has become increasingly desirable. Tall oil, derived from pine wood and consisting of approximately equal amounts of fatty and resin acids plus 6 to 10% of nonvolatile matter, is a by-product of the alkaline sulfate pulp process.

Chapman, Hastings, and Pollak (5) recently reviewed this subject and presented data on the application of the Wijs method to tall oil. Their studies of the effects of temperature, excess iodine, and absorption time on the reaction showed that the resulting iodine values were markedly affected by these variables. Boesecken and Gelber (4) claimed that the Wijs method was not satisfactory for conjugated systems and that aromatic compounds interfered with the iodine absorption. Kutsch, Wagner, and Zuravlev (14) tried several methods for determining the iodine number of nonfatty materials of high molecular weight, such as turpentine and rosin oil. These methods were all very sensitive to time of reaction and sample weight. Jones and Neville (9) obtained similar results with the Wijs method on conjugated drying oils. They suggested the use of the Wijs determination as a method of qualitatively indicating the presence of conjugated double bonds because of its extreme sensitivity to sample weight in this case. Dittmer (8) reported difficulties in iodine number determinations of tall oil and noted that the results showed deviations from the iodine values of fatty acids, which, he explained, were caused by a polymerization phenomenon. An exhaustive discussion of the variables involved in iodine number determinations is presented in a paper by Margosches (15).

From the wide variety of iodine value ranging from 100-210 reported for tall oil by many investigators (5) and the experience

of the authors with the Wijs method (which is in substantial agreement with that of Chapman, Hastings, and Pollak), it was obvious that no satisfactory and practical iodine number method for tall oil was available. Most of the compounds present in tall oil are highly unsaturated and are thought to be conjugated to an appreciable extent. They do not respond easily to ordinary methods of halogen addition.

Von Mikusch and Frazier (26, 27) recently recommended the use of Hanus' solution, in which the concentration of iodine bromide is about doubled, for determining the total unsaturation of oils and fatty acids containing conjugated double bonds. Using 1-hour absorption at 20° C. and 500 to 800% excess reagent they found the iodine value of distilled tall oil to be about 204. This procedure, applied to crude tall oil, has not been tried by the authors, since apparently consistent results were obtained by the method described in this paper.

The advantages of using a more active halogen as the addition reagent and the possible use of a catalyst to promote the reaction were immediately apparent. Rosenmund, Kuhnhen, Rosenberg-Gruszynski, and Rosetti (22) developed a bromine addition method using pyridine sulfate dibromide ( $C_5H_5N \cdot H_2SO_4 \cdot Br_2$ ) in glacial acetic acid solution approximately 0.1 N with respect to bromine. Except for the use of this reagent, the general procedure

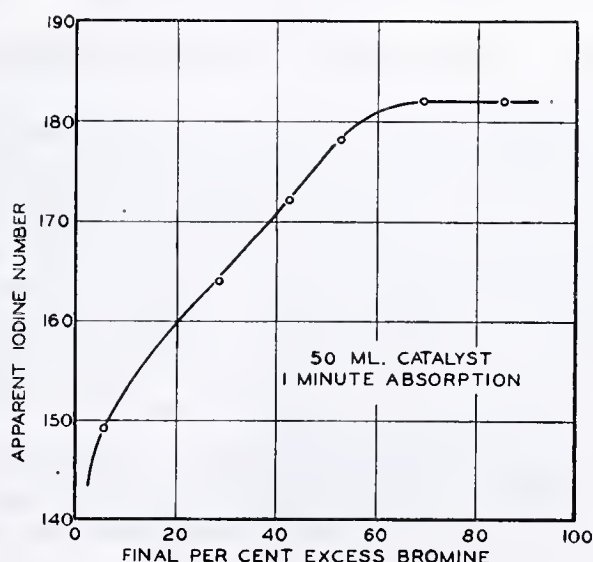


Figure 1. Effect of Excess Bromine on Iodine Number of Crude Tall Oil for Short Absorption Period

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ture is essentially the same as that of the Wijs method. The Rosenmund-Kuhnnehn method applied to oleic acid and cholesterol was tested and discussed by Yasuda (29), Dam (6, 7), and Page and Rudy (18). After comparative tests with various reagents Govindarajan (10) concluded that the Rosenmund-Kuhnnehn method was the most satisfactory for determining the iodine numbers of linseed, sunflower, and croton oils.

Rosenmund and Kuhnnehn also discussed further applications of their method (21) and the chemistry of pyridine sulfate dibromide (20). They showed that the compounds of bromine with pyridine or quinoline are active bromine addition agents and that they do not participate in the secondary reactions of substitution or oxidation. The solution is very stable over a long period of time and is much easier to prepare than the Hanus, Hübl, and Wijs solutions.

The use of a catalyst to promote absorption has been reported by several investigators. Hübl (12) suggested using mercuric chloride with an iodine-alcohol solution and Wijs (28) also reported his investigations in this connection. More recently Scotti (24) proposed the use of mercuric acetate, dissolved in glacial acetic acid, with an iodine-benzene reagent and claimed accelerated absorption by this method. Hoffman and Green (11) proved the superiority of mercuric acetate as a catalyst but preferred to use it with the Wijs solution. Mercuric acetate has also been tested with an elemental bromine-acetic acid reagent by Jaspersen (13) and with the Hanus and Wijs reagents by Norris and Buswell (17).

#### DEVELOPMENT OF METHOD

In addition to the official Wijs iodine number procedure (1, 2, 3), the methods involving the use of mercuric acetate catalyst with the Wijs solution (11) and of pyridine sulfate dibromide reagent without a catalyst (22) were tried on crude tall oil. In each case, these procedures were unsatisfactory because of the extreme sensitivity of the results to the amount of excess reagent and the time of absorption.

Preliminary tests, in which mercuric acetate catalyst was used with the pyridine sulfate dibromide reagent, gave greatly improved results, however, and the most favorable conditions for carrying out the reaction were then determined.

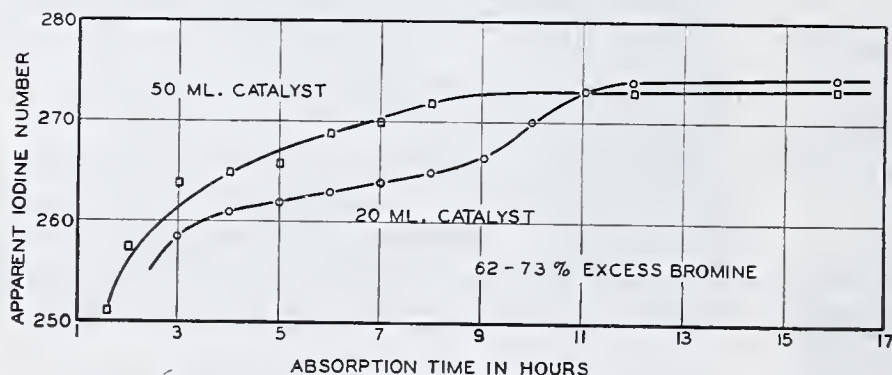


Figure 2. Effect of Absorption Time on Iodine Number of Crude Tall Oil

**GENERAL PROCEDURE.** In each series of determinations carbon tetrachloride was used as the solvent for the crude tall oil. The weighed sample in solution was allowed to react for a definite time at  $27 \pm 1^\circ \text{C}$ . with an excess of the pyridine sulfate dibromide reagent in the presence of mercuric acetate catalyst. The exact total reagent was known by the usual iodometric titration of a blank with aqueous 0.1 *N* sodium thiosulfate solution. After absorption, the remaining reagent was determined by a similar titration, and the difference between the two titrations was equivalent to the amount of halogen absorbed by the sample. The iodine number was then computed in the usual manner. The catalyst used for all determinations was a 2.5% solution of mercuric acetate in glacial acetic acid.

During preliminary runs it was noted that the presence of light during absorption affected the results to a slight degree. Therefore, as a precautionary measure, the absorptions were allowed to take place in the dark and the pyridine sulfate dibromide reagent was stored in an amber bottle.

For these reactants at a fixed temperature, the degree of absorption obtained is a function of the amount of catalyst, the

length of the absorption period, and the amount of excess reagent. For a fixed sample weight and a fixed original amount of reagent, the total excess reagent remaining after absorption will, of course, depend on the extent of the reaction. Hence, it is difficult to fix this variable absolutely, as, for example, when the effect of absorption time is being studied. The relatively slight variation, however, did not appear to affect the findings adversely.

Before studying the effect of absorption time it was desirable to obtain some idea of the amount of excess reagent necessary for a series of determinations was made with 50 ml. of catalyst solution for a short absorption period of one minute, using varying sample weights (Figure 1). Although the maximum iodine values obtained are low because of the short absorption period, the effect of per cent excess reagent disappears at values of about 60-70%.

**EFFECT OF ABSORPTION TIME.** Using 62 to 73% excess reagent, two series of determinations were made (Figure 2). With 20 ml. of catalyst solution, absorption is complete in about 12 hours. When 50 ml. of catalyst solution are used, the reaction is complete in 9 to 10 hours. The initial absorption is comparatively rapid and from this fact one might deduce that the remaining slow absorption is by conjugated groups. A slight dilution effect is noticeable in Figure 2, where the larger volume of catalyst solution gives the lower iodine value. In preceding work without the use of a catalyst, an absorption time of 75 hours was necessary to obtain an iodine value of 275 with no further absorption after 96 hours.

**EFFECT OF EXCESS REAGENT.** In order to determine if excess reagent could be used with a longer absorption period, a series of analyses was made using 20 ml. of catalyst and 16 hours reaction time. The results are plotted in Figure 3. The iodine value rises with increasing per cent of excess reagent and becomes constant at values above 50%. It can be concluded that 50% excess is the minimum amount of reagent with which reproducible results can be obtained under the above conditions.

#### SUGGESTED ANALYTICAL METHOD

**REAGENTS.** Aqueous starch indicator solution prepared by adding 10 grams of soluble starch to 100 ml. of boiling water. Add 10 grams of mercuric iodide to prevent bacterial action (19). The mercuric iodide is very sparingly soluble and will completely settle after standing about an hour.

Aqueous 15% potassium iodide solution. This solution will decompose and turn a pale yellow after standing for a long period. When necessary the color can be discharged by the addition of one drop or two of 0.1 *N* sodium thiosulfate.

Mercuric acetate in glacial acetic acid, 2.5% solution. Standardized aqueous 0.1 *N* sodium thiosulfate. Carbon tetrachloride.

Pyridine sulfate dibromide solution. Place 10 ml. of glacial acetic acid in each of three 100-ml. Erlenmeyer flasks. To the first add slowly 1.0 gram of pyridine with cooling. In the same manner add  $20 \pm 0.2$  grams of concentrated sulfuric acid to the second flask. When cool, combine these solutions with further cooling, by adding the sulfuric acid-acetic acid mixture to the pyridine solution. To the third flask add carefully 1.0 gram of bromine. Now add this solution to the mixture of the first two solutions and transfer to a 1-liter volumetric flask. Make up to the mark with glacial acetic acid and transfer to a 500-ml. amber or black-painted 2.5-liter glass-stoppered storage bottle. Add 1000 ml. of glacial acetic acid and mix the solution thoroughly.

**PREPARATION AND WEIGHING OF SAMPLE.** Run all determinations in duplicate.

Transfer a sample of the approximate weight noted below to a beaker in which it can be warmed if necessary to a  $50^\circ \text{C}$ . glass-stoppered bottle containing 25 ml. of carbon tetrachloride. To prevent possible loss of volatile halogen during the absorption period, use is recommended of the special iodine flasks with a seal around the top to provide a liquid seal of potassium iodide solution. Weighing is accomplished by difference.



The following maximum sample weights are suggested for various oils in order that the proper amount of excess reagent will be present during absorption:

Approximate Iodine No.	Maximum Sample Weight, Grams
275	0.115 (about 3 drops)
100	0.250 (about 8-10 drops)
5-10	2-3 (about 100 drops)

For determinations on individual samples of highly unsaturated oils, it is desirable to make up 500 ml. of carbon tetrachloride solution in a volumetric flask and remove a 25-ml. aliquot portion for analysis which permits larger sample weight. This procedure is more accurate, because the error inherent in weighing by difference a very small sample of heated oil is reduced. However, this method requires large volumes of solvent and excess apparatus when a large number of routine determinations must be run.

**ABSORPTION AND TITRATION.** Run a blank with each series of determinations. To the weighed sample in carbon tetrachloride solution, add exactly 50 ml. of pyridine sulfate dibromide solution from a pipet (use water suction). Now, add 20 ml. of mercuric acetate catalyst. Allow the bottle to stand in a dark place 16 hours at uniform room temperature. Add 20 ml. of 15% aqueous potassium iodide solution, allow to stand 1 minute, and then add 100 ml. of distilled water. Titrate in the usual manner with standardized 0.1 *N* sodium thiosulfate, using starch solution as the indicator. The end point should persist for 2 minutes. Equivalent results can be obtained with 10 hours' absorption using 50 ml. of mercuric acetate catalyst solution.

It is necessary to run blank and sample determinations at the same time and to use the same amount of catalyst in each case. A crystalline deposit of the catalyst is usually observed after the samples have stood for some time, probably because the solubility of mercuric acetate in acetic acid is depressed slightly when carbon tetrachloride is present. However, this phenomenon apparently has no adverse effect on the results. Calculations for the iodine number are performed in the usual manner.

#### PRECISION OF THE METHOD

A series of twenty determinations using 20 ml. of catalyst and 16 hours' absorption time was run on individually weighed samples of crude tall oil taken from the same representative lot. The results were then statistically analyzed according to methods suggested by Scarborough (23) and Mellor (16). The most probable value of the iodine number of the particular sample was found to be  $274.68 \pm 2.09$ . The probable error of a single observation was  $\pm 2.09\%$ . The iodine number of crude tall oil will, of course, vary somewhat, depending upon the source of the oil. Values for eight different commercial samples of crude tall oil were found to vary between 237 and 287.

Results obtained by an experienced analyst on individually weighed duplicate samples of tall oil which are within 5 or 6 iodine number units of each other may be regarded as good checks. Doubtless, the chief error lies in the weighing, because the sample must necessarily be small for such a highly unsaturated substance. It is difficult to weigh warm oil samples by the difference method with good accuracy, and a direct method of weighing with the use of microbeakers should permit some improvement.

#### ABSENCE OF SECONDARY REACTIONS

If secondary substitution or oxidation reactions took place along with addition to the double bonds, hydrobromic acid would be present in the reaction mixture after absorption. A sample of crude tall oil, along with a blank, was allowed to stand 23 days in the presence of an excess of the pyridine sulfate dibromide re-

agent and 20 ml. of the mercuric acetate catalyst solution. Distilled water was added and the free bromine was then removed by successive extractions with carbon tetrachloride. Acidification of the water layer with sulfuric acid and subsequent oxidation with hydrogen peroxide gave a negative test for bromide ion, using the sensitive fluorescein method of detection (25). This result is evidence of the absence of a secondary substitution reaction and substantiates the claim of Rosenmund and Kuhnnein (20) in this respect.

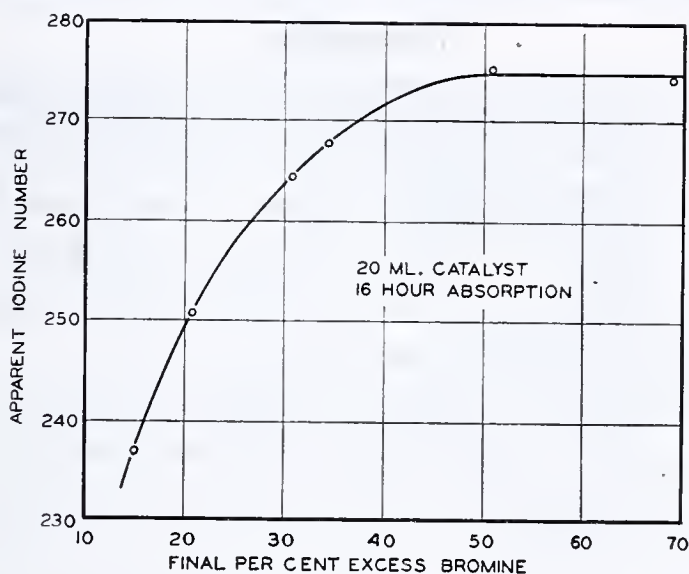


Figure 3. Effect of Excess Bromine on Iodine Number of Crude Tall Oil for Long Absorption Period

The reported iodine value does not necessarily represent with great accuracy the true total carbon-to-carbon unsaturation of crude tall oil. Reactions other than carbon-to-carbon addition or the secondary substitution, shown above to be absent, although improbable, may occur and cause the apparent iodine value to be higher than the true one for carbon-to-carbon unsaturation alone. However, the results obtained appear to be consistent, and the method proposed has distinct value for purposes of identification and analytical control. For example, the degree of hydrogenation of a partially hydrogenated pilot-plant sample of crude tall oil was computed from iodine numbers determined by this method. The result agreed almost exactly with that obtained by graphical integration of the differential rate curve, determined experimentally by observations of hydrogen pressure drop.

#### DISCUSSION

Application of this method to other fatty acids and glycerides immediately suggests itself. Because of its success with tall oil, it is especially recommended for other highly unsaturated and conjugated systems such as rosin acids, tung oil, linseed oil, natural resins, synthetic rubberlike materials, etc.

Although the effect of temperature on the rate of bromine absorption was not studied, this effect undoubtedly does exist. It is therefore necessary that the absorption be allowed to take place at constant temperature. When a large number of routine determinations are made, it is suggested that during the absorption period the sample bottles be placed on a rack, provided with a cover or hood to keep out the light, and partly immersed at constant temperature in a thermostatically controlled water bath. It is possible that an absorption temperature somewhat higher than  $27^\circ\text{C}$ . may shorten the required absorption period.

Further refinements of this method suggest themselves, such as increasing the concentration of bromine in the reagent, leading to the use of either less volume of reagent or a greater quantity of sodium thiosulfate solution for the titrations, the use of auto-



matic pipets and highly accurate thin-bore burets, and the development of a micromethod. The reader is referred to von Mikusch and Frazier (26) and Yasuda (29) for specific examples.

#### ACKNOWLEDGMENT

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## An Improved Vacuum Distilling Head

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ONE of the most convenient setups for ordinary vacuum distillation consists of a Claisen flask in conjunction with a distilling flask or another Claisen flask as a condenser and receiver. This arrangement has several advantages. It may be set up and taken down rapidly; a good vacuum can be readily obtained; there is no appreciable holdup of the distillate between still and receiver; and, after the distillation, the distillate is in a still ready for distillation again if need be. On the other hand, the necessity of keeping on hand many different sizes of flasks is a serious disadvantage, since these flasks have little use except for distillations.

The apparatus in Figure 1 was designed to overcome this disadvantage, and also for convenience in setting up the still without sacrificing any of the advantages of the Claisen flask.

Openings 3, 4, and 8 are for capillary tube, thermom-

eter, and vacuum, respectively. Standard-taper joints at 4 and 7 greatly facilitate change of flasks 1 and 5. Drip tip 6 prevents holdup in the delivery tube. Flask 5 is cooled with running water or a bath of ice or solid carbon dioxide. No other condenser is needed.

The flasks, when not used for distilling, may be used for other purposes. The distilling head is simple to construct and takes up very little space. All the advantages of the Claisen flasks are retained without the need of a large stock of flasks which have only one use. With this apparatus it is practical to use flasks of 50- to 2000-ml capacity without altering the dimensions of the head. A number of laboratory supply houses make standard-taper Claisen type distilling head, but in order to use their apparatus for a vacuum distillation either an auxiliary condenser or a vacuum adapter is necessary, which increases the holdup and complexity of the apparatus.

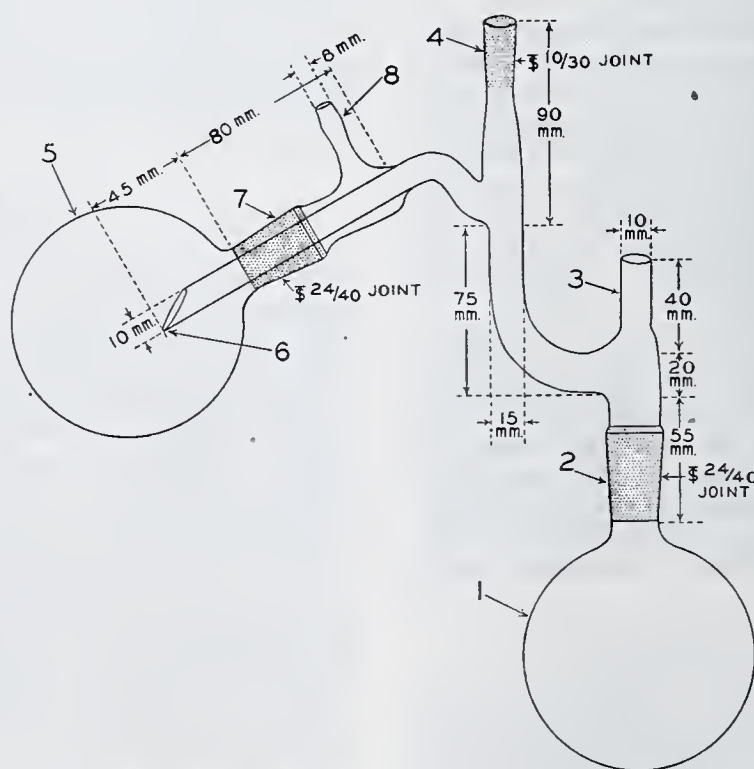


Figure 1. Vacuum Distilling Head



# Colorimetric Determination of Nickel in Steel

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The method of Murray and Ashley for the colorimetric determination of nickel in steel is outlined and criticized. Experimental data are presented on the stability under various conditions of the red color of oxidized nickel dimethylglyoxime. A modification of the method is described which gives a highly stable and readily repro-

MURRAY and Ashley (1) have presented a method for developing quantitatively the red color of oxidized nickel dimethylglyoxime.

The sample is dissolved in dilute nitric acid (in the case of difficultly soluble chromium-nickel steels, a mixture of nitric and hydrochloric acids is used). To an aliquot of suitable size the following additions are made: citric acid to prevent iron precipitation at the final pH, bromine water to oxidize nickel, ammonium hydroxide in sufficient quantity to bring the pH to 8-9, and dimethylglyoxime in the form of 1% solution in alcohol to develop the red nickel color. The solution is diluted to known volume and the color is compared. A wave length that has been recommended for spectrophotometric reading is 530 m $\mu$ .

Although the method of Murray and Ashley has been used with some success in this laboratory and elsewhere, it has certain undesirable characteristics. The color developed in the nickel solution shows continuous change from the instant of addition of dimethylglyoxime, tending for the first few minutes to become more intense, then to fade. Murray and Ashley note the phenomenon of the color intensity change at 530 m $\mu$  with time, and present a series of curves illustrating its characteristics. At no time is there a period of constant transmittance in the transmittance *vs.* time curve of sufficient duration to give a time margin for truly reproducible reading.

Both the slope and the time of the minimum of the transmittance *vs.* time curve appear to be changed by changes in the nickel concentration of the solution used for making the curve. Small differences in pH at the time of adding dimethylglyoxime to otherwise similar solutions were found to have pronounced effects on the shape of the transmittance *vs.* time curve, altering both the slope and the position of the minimum to a marked degree.

If 80% of the recommended amount of ammonium hydroxide is used, no color at all develops (pH 6.8). (All pH measurements were made with a Leeds & Northrup potentiometric pH meter using a standard glass electrode and a calomel-saturated potassium chloride reference electrode.) With 90% of the amount required (pH 8.1), the rate of color development is slow and rate of fading is high. If the concentration of ammonium hydroxide is high, the rate of color development is high and the rate of fading is low, solutions retaining substantially all their color for several hours. If as much as four times the recommended amount of ammonium hydroxide is used, the color is completely developed in about 30 seconds. A second effect enters, however—a rapidly increasing interference of the iron in the solution. Ultimately iron hydroxide is precipitated under these conditions. Critical examination of the pH range within which the Murray-Ashley method is workable shows it to be less than 1 pH unit, pH 8.2 to 9. It was found that the amounts of tartaric acid, bromine water, and dimethylglyoxime solution were not critical above a minimum value if sufficient extra ammonium hydroxide was added to neutralize increases in the acid content.

It was concluded that the unmodified method of Murray and Ashley, while suitable for many purposes, is not sufficiently reproducible (at least without elaborate precautions) to meet the needs of this laboratory. It does, however, have the virtue of being rapid. It was in an effort to retain its speed while improving its accuracy that this research was undertaken. The original method recommends dissolving the sample in 1 to 1 nitric acid,

developing a red color, and is particularly suitable for routine work because of its rapidity and manipulative simplicity. Copper and cobalt interfere only slightly; the other elements ordinarily found in steel do not interfere. The accuracy of the method is comparable to that of routine gravimetric procedures.

except difficultly soluble chromium-nickel steels for which a mixture of equal parts of nitric and hydrochloric acids is suggested. Since many of the steels analyzed in this laboratory contain about 1% chromium and do not dissolve completely in nitric acid or rapidly in hydrochloric acid, a method was tried of decomposing first in an "acid mixture" (133 ml. of 1.82 sp. gr. sulfuric acid and 167 ml. of 85% phosphoric acid per liter of solution) and then completing dissolution by adding 1 to 1 nitric acid. For 1% chromium steels the method was more rapid than decomposing with hydrochloric acid and more nearly complete than dissolving in nitric acid. No effect on the development of the nickel color was observed.

In the effort to produce a nickel color that would be quickly formed, stable, and reproducible without elaborate control of the conditions of development, various other basic substances were tried in place of ammonium hydroxide. Among them were sodium carbonate, sodium tetraborate, sodium orthophosphate, potassium pyrophosphate, and sodium hydroxide, all in the concentrations required to produce the proper pH range for color development. None proved satisfactory. Other oxidizing agents such as sodium perborate, potassium chlorate, potassium iodate, and hydrogen peroxide were tried in place of the bromine. Only ammonium peroxydisulfate in the presence of silver ion showed promise, but the desired degree of color stability was not achieved with it. The effects of solution temperature on color development were studied, but no worthwhile modification utilizing temperature control was found. Color development at high ammonium hydroxide concentration followed by reduction of the solution pH to 8.5 to prevent iron precipitation proved unworkable.

It was realized at this point that tartaric acid will hold iron in solution at a considerably higher pH than will citric acid. Accordingly, the citric acid was replaced by tartaric. Upon experiment it was discovered that, while iron will develop an interfering color in tartrate solutions made basic with ammonium hydroxide nearly as quickly as in citrate solutions under the same conditions, the interference and any precipitate that may form can be cleared up rapidly by increasing the pH still further with sodium hydroxide. This is not true of citrate solutions. While the nickel color develops slowly and incompletely in very dilute sodium hydroxide solutions of pH 8 to 9 and not at all in more concentrated solutions, the color, once developed, remains stable over long periods of time even in rather strongly basic sodium hydroxide solutions. In view of these facts, the following approach was tried:

The sample was treated with tartaric acid and bromine and made strongly ammoniacal. Then the dimethylglyoxime solution was added. Under these conditions the color developed rapidly. After 1 minute, sodium hydroxide was added. After 2 to 3 minutes the increase in iron interference which had taken place in ammoniacal solution cleared up completely and the color became stable. No further change in color and consequently in transmittance at 530 m $\mu$  was observed. The reading at 24 hours was identical with the reading 5 minutes after adding dimethylglyoxime.

## PROCEDURE

Since these results seemed very encouraging, a procedure was devised to make use of them and was used in all the studies hereafter reported. High-purity reagents must be used. Particular



attention should be paid to the suitability of the tartaric acid and of the dimethylglyoxime.

Decompose a 0.25-gram sample in 20 ml. of an acid mixture (133 ml. of 1.82 sp. gr. sulfuric acid and 167 ml. of 85% orthophosphoric acid per liter of solution). Steels containing little chromium may be dissolved directly in 8 *N* nitric acid. Stainless-type steels may require the use of hydrochloric acid. Cautiously add 10 ml. of 8 *N* nitric acid and boil to expel oxides of nitrogen. Transfer the solution to a volumetric flask of suitable size and dilute to the mark. Transfer by pipet to a 100-ml. volumetric flask an aliquot of the diluted solution containing between 0.05 and 0.3 mg. of nickel. Add to it, mixing after each addition, 5 ml. of a 20% tartaric acid solution, 5 ml. of saturated bromine water, 10 ml. of 0.90 sp. gr. ammonium hydroxide, and 5 ml. of a 1% solution of dimethylglyoxime in methyl alcohol. (Occasional difficulties in development of color and in fading upon addition of sodium hydroxide have been traced to impure or partially decomposed tartaric acid and dimethylglyoxime. C.P. reagents are not uniformly satisfactory in this respect. Impure tartaric acid interferes with development of color upon addition of dimethylglyoxime, in extreme cases preventing any color formation at all. Impure dimethylglyoxime results in a fading of the color upon addition of sodium hydroxide. The fading may take place very rapidly or slowly, depending on the degree of impurity. Both difficulties can be overcome by special treatment. Addition of a second 5-ml. portion of bromine water after introduction of the dimethylglyoxime ensures complete color development. Difficulties with the dimethylglyoxime reagent can be overcome by acidifying the alcohol solution with dilute sulfuric acid and adding enough bromine water to color it yellow. This should be done 15 to 30 minutes before it is used. Occasional small further additions of bromine water are necessary to keep the solution yellow. The treated reagent is usable for only a few hours.)

After 1 minute add 10 ml. of 6 *N* sodium hydroxide solution and dilute to the mark. After 5 minutes, transfer the solution to the optical cell and compare the transmittance at 530  $m\mu$  with that of pure water.

Two transmittance *vs.* wave-length curves were prepared, one from National Bureau of Standards Bessemer steel 10d containing substantially no nickel, and one from a nickel solution made from C.P. nickel nitrate. They are shown in Figure 1 as read on a Coleman Universal spectrophotometer. A Beckman spectrophotometer, with a much narrower wave band than the Coleman, gave a similar curve for the nickel solution; the positions of the maxima and minima were identical. Appreciable interference of the blank (Bessemer steel), however, did not occur on the Beckman instrument until wave lengths as short as 470  $m\mu$  were reached.

From a study of these curves it was decided that the most satisfactory wave length for reading the transmittance of the nickel color in steel on a Coleman Universal spectrophotometer, which has a wave band width of 35  $m\mu$ , is 530  $m\mu$ , the wave length originally suggested for the method of Murray and Ashley. This selection was made on a basis of minimum interference of the blank and maximum interference of the nickel color. For instruments with a wave band much narrower than 35  $m\mu$ , such as the Beckman photoelectric spectrophotometer, a lower setting such as 480 or 490  $m\mu$  seems to be preferable, since the lower value is closer to a minimum of the nickel curve and since iron interference is small in this range with such an instrument. Presumably, it would be possible to use for both spectrophotometers a wave-length setting at the 470  $m\mu$  minimum of the nickel curve, if a blank prepared from a steel free of nickel were used as the reference solution, thus canceling the effect of iron interference.

For colorimeters using filters one probably would do best to select a filter with a rather sharp cutoff at about 450  $m\mu$ , passing no light of shorter wave length. This selection is indicated to eliminate interference due to the color of the iron present. Increased sensitivity can be obtained by further restriction of the wave length of light used for comparison to the range of maximum interference of the nickel color.

Samples were taken from a series of National Bureau of Standards nickel-containing steels and mixtures of these steels to cover in small steps a series of nickel percentages from 0.002 to 5.12.

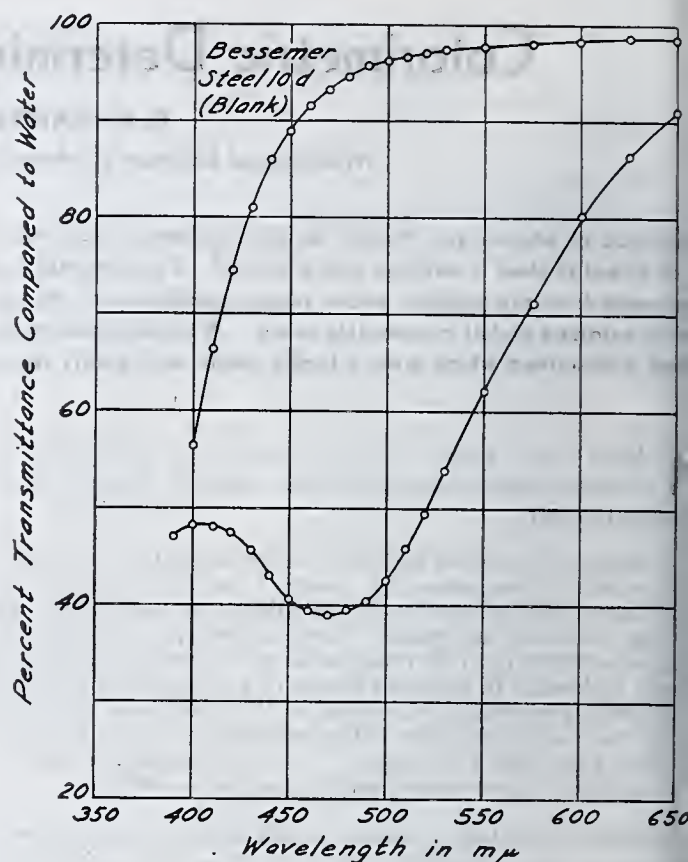


Figure 1. Per Cent Transmittance *vs.* Wave Length  
Curves for Bessemer steel blank and C.P. nickel nitrate solution as read on Coleman Universal spectrophotometer (35  $m\mu$  band width)

They were prepared according to the method previously outlined, and the points so obtained were plotted on semilog paper with the per cent transmittance of the sample compared with water at 530  $m\mu$  as the ordinate and the per cent of nickel in steel as the abscissa. In this fashion three transmittance-concentration curves were obtained, for 0 to 1%, 1 to 2.5%, and 2.5 to 5.5% nickel steels. A total of 27 concentrations was used in obtaining the three curves.

The color was found to follow the Beer-Lambert law very closely when read at 530  $m\mu$ ; therefore the transmittance *vs.* concentration curves, when plotted as described, were straight lines over the entire range utilized.

No one of the 27 points determined from the National Bureau of Standards samples deviated from the transmittance *vs.* concentration curves plotted from them by more than 2% of the total nickel present in the steel in the case of steels containing more than 1% nickel. No one of the points deviated from the transmittance *vs.* concentration curve by more than 0.02% nickel ("points") based on the total analysis of the steel in the case of steels containing less than 1% nickel. These points were established by single determinations, not by averages of groups of determinations. The differences between the high and the low values on the National Bureau of Standards reports sent with each steel are as great as 1.7% of the total nickel present in the steel in the case of steels containing more than 1% nickel. Differences of as much as 0.021% of nickel (2 "points") based on the total analysis of the steel are listed in the case of steels containing less than 1% nickel.

Reproducibility was checked by making five complete determinations by the authors' method on National Bureau of Standards nickel steel 33b containing 3.48% nickel. Using the transmittance *vs.* concentration curve established for steels containing 2.5 to 5.5% nickel, the average value of the five determinations was 3.48% nickel. The maximum deviation from the average value was -0.05% nickel. High and low values on the National Bureau of Standards report are 3.51 and 3.46% nickel, respectively. The method appears to be capable of the high order of reproducibility necessary for precise steel analysis.



SENSITIVITY

Tests were made to determine the sensitivity of the method to all variations in procedure. It was found that the tartaric acid, bromine water, and dimethylglyoxime added could be decreased 25% or increased 100% without affecting the results. The ammonium hydroxide may be decreased 20% or increased 50% without effect. Approximately the same range of values holds for the sodium hydroxide. It was determined that the time elapsed between adding the dimethylglyoxime and the sodium hydroxide is not critical so long as it exceeds 1 minute—color development is complete in less than 1 minute.

Three identical samples equivalent to a standard steel containing 0.60% nickel were prepared, using for each sample 0.125 gram National Bureau of Standards Bessemer steel 10d and 0.125 gram of nickel-chromium steel.32c. To sample 1 the sodium hydroxide, which arrests color development as well as preventing precipitation, was added 30 seconds after adding the dimethylglyoxime; to sample 2, 5 minutes after adding the dimethylglyoxime; and to sample 3, 10 minutes after adding the dimethylglyoxime. In each case the solution was diluted to the volumetric mark and mixed immediately after adding the sodium hydroxide. Transmittance readings in each case were taken 10 minutes after adding the sodium hydroxide. The results, expressed in terms of the analysis of the steel as read from the 0 to 1% nickel curve, averaged 0.599% nickel with a maximum deviation from the average value of 0.005% nickel.

INTERFERENCES

The method was tested for interference by copper, cobalt, tungsten, molybdenum, chromium, and vanadium. The small amounts of copper (less than 0.2%) present in the usual steels did not interfere. Copper, when present to the extent of 0.50% in the steel, caused a positive nickel error of 0.02%. Cobalt, when added to the sample equivalent to 2.5% in the steel, caused a positive error of 0.03% nickel. Both elements were tested for interference on a sample of steel containing 0.60% nickel. The inter-

Table I. Analysis of Steels

	Sample 50a		Sample 132	
	Bureau of Standards	Authors' method	Bureau of Standards	Authors' method
	%	%	%	%
Tungsten	18.25	..	6.29	..
Chromium	3.52	..	4.09	..
Vanadium	0.970	..	1.64	..
Nickel	0.045	0.07	0.095	0.13
Molybdenum	..	..	7.08	..

ference of the other elements mentioned was determined by using the method without modification of any sort to analyze for nickel in two Bureau of Standards tool steel samples, 50a and 132.

In view of the extreme conditions in these two analyses and the small error in nickel in each case, it was concluded that these elements do not interfere with the determination to any appreciable extent. In the analysis of ordinary chromium-nickel stainless steels, no interference by chromium was observed.

ADVANTAGES OF METHOD

The most important advantages of the method are its rapidity and its freedom from manipulations requiring exceptional precautions or a high degree of analytical skill. It is well suited for routine use. When a group of five samples was analyzed by a worker only recently familiar with the method, the total elapsed time, exclusive of weighing, was 1 hour and 10 minutes (results reported above in paragraph on reproducibility). If a large number of samples is run at one time, one thoroughly familiar with the method can reduce the time required per sample to about 8 minutes.

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# Identification of Nornicotine in Tobacco

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THE rather common occurrence of nornicotine in tobacco (3, 4, 6, 7) indicates the need of a reliable qualitative means for identifying this alkaloid when present with nicotine.

Shmuk (8) identified nornicotine in such a mixture after extracting the ether-soluble material from alkalized plant material, by forming the alkaloid picrates, recrystallizing from hot water, and methylating the mixture of picrates. He attributed the resulting elevation in melting point of the picrate to the conversion of nornicotine to nicotine.

This procedure has several serious drawbacks. The ether extract of the alkalized plant material will contain pyridine and other amines, not all of which are removed by "blowing". These compounds as well as the alkaloids form picrates. The recrystallizations that occur before and during methylation will tend to remove these amine picrates but at the same time to eliminate the picrate of the minor alkaloid. If the minor alkaloid is present in small amount, it may be lost in the recrystallization of the original picrate. Since the mixed picrates before methylation have a melting point lower than that of nicotine picrate, and, being solid, require the addition of liquid for the methylation, it is possible for a fractional crystallization to occur with the formation of new picrate crystals richer in nicotine content and consequently having a higher melting point. Such a rise in melting point could easily be misinterpreted as being due to methylation of nornicotine, while in fact the alkaloid

picrate originally present may have been simply rendered richer in nicotine by recrystallization. Such a recrystallization proves neither the presence nor the absence of nornicotine.

The proposed method differs from that of Shmuk in the isolation of the alkaloids and in the position of the methylation step. The steam-volatile tobacco alkaloids are separated from all other amines and methylated before the picrate is formed. The melting points of the picrates formed are compared before and after methylation. Since they are not recrystallized no fractional crystallization can occur.

Markwood (5) introduced methylation as a step in the determination of nornicotine, but since his method did not depend on melting points, he failed to report the melting point of methylated nornicotine picrate as an indication of the formation of nicotine.

The method presented here is based on the quantitative methylation of the nornicotine and also on the elevation of the melting point of the alkaloid picrate. Consequently, the methylation of nornicotine to nicotine was investigated. The nornicotine used in preparing the standard solution was identical with that used by Markwood (7), had been isolated from Robinson's Medium Broadleaf, a strain of Maryland tobacco, and formed a picrate melting at 190-191° C. The absence of nicotine was established by finding no alkaloid in the distillate after treating a sample with nitrous acid, making the aqueous solution just basic to phenolphthalein, and steam-distilling.



Table I. Effect of Methylation of Nornicotine on Picrate Melting Point

Sample	Nornicotine in Alkaloid Mixture %	Picrate Melting Point <sup>a</sup>		
		Original ° C.	Methylated ° C.	Mixed ° C.
Nicotine Nornicotine Mixtures	100	221-223 187-190	221-223	220-223
1	94.8	168-180	220-223	221-223
2	89.4	180-184	223-224	221-223
3	84.3	175-183	222-223	221-223
4	79.2	178-183	222-223	220-221
5	22.9	205-214	222-224	221-224
6	19.2	210-218	222-224	222-224
7	13.7	213-220	222-224	222-224
8	4.6	215-223	222-224	222-224

<sup>a</sup> Not recrystallized. Melting points corrected and rounded off to whole numbers.

## METHYLATION OF NORNICOTINE

Ten milliliters of the nornicotine solution containing 23.2 mg. of the alkaloid were treated with formic acid and formaldehyde according to Markwood's method (5), made alkaline to phenolphthalein, and steam-distilled. No alkaloid was detected in the distillation residue.

The alkaloid in the distillate was precipitated as a picrate, the melting point of which, when not recrystallized, was 221-223° C. A mixed melting point with nicotine picrate prepared from a sample of pure nicotine showed no depression. The pure nicotine had been treated with nitrous acid for the removal of nornicotine. The absence of alkaloid in the distillation residue and the melting point of the picrate of the alkaloid in the final distillate prove the quantitative methylation of nornicotine to nicotine by means of formaldehyde and formic acid. A sharp melting point is not to be expected after methylation when no recrystallization has been made. In obtaining the melting points, consideration must be given to the spread, although the upper limit is the most easily observed temperature. The entire spread must be considered as the melting point. Values obtained with known mixtures of nicotine and nornicotine are shown in Table I.

## PROCEDURE

A 1-gram sample of tobacco, 10 ml. of sodium hydroxide (30% by weight), and 10 grams of sodium chloride are steam-distilled into 3 ml. of dilute hydrochloric acid (1 + 4) until a fresh portion of distillate gives no opalescence when a few milliliters are tested with silicotungstic acid solution. The volume of distillate is about 100 ml. The steam-volatile alkaloids are separated from other picrate-forming materials by precipitation by silicotungstic acid solution (12%) according to A.O.A.C. procedure (1), filtered on a small, hardened filter paper in a Hirsch funnel, and washed with water containing 1 ml. of concentrated hydrochloric acid per liter. The alkaloid in this residue is steam-distilled from sodium hydroxide and sodium chloride into hydrochloric acid, as above. The distillate is concentrated to about 10 ml. and divided into two approximately equal portions. One portion is methylated according to Markwood's method by adding 2 drops of formic acid and 5 ml. of formaldehyde (37%) and refluxing for 15 minutes. It is then cooled and neutralized, and 25 ml. of saturated aqueous picric acid solution are added. This solution is next concentrated by boiling to about 30 ml. and allowed to cool slowly. Twenty-five milliliters of the picric acid solution are added to the neutralized unmethylated portion, warmed to dissolve the precipitate, cooled slowly. The alkaloid picrates are filtered off and washed, once with dilute picric acid solution and once with water. The melting points of the picrates of the unmethylated distillate, the methylated portion, and the methylated portion mixed with nicotine picrate are compared for an elevation of temperature due to methylation. The nicotine picrate should be prepared from pure nicotine and washed in the same manner as the unmethylated portion.

If the melting point of the methylated portion is higher than that of the untreated portion and is comparable with that of the nicotine picrate, and no depression occurs in the mixed melting point, nornicotine is confirmed. Since nornicotine is the only alkaloid that can be methylated to form nicotine and no derivative of other alkaloids would affect the mixed melting point, it is evident that nornicotine is the only steam-volatile alkaloid aside from nicotine which is present in the tobacco test. Table II shows the effect of methylation.

Nornicotine in tobacco, tobacco mixtures, and nicotine preparations may be identified in the same manner, although small samples should be used when the alkaloid content is high.

Although this method appears to be lengthy, at present it offers the best chemical means for the identification of nornicotine, can be conducted in any laboratory, and does not require expensive and specialized equipment, such as a polarimeter which is now either not available or difficult to obtain. It offers the following advantages over the method proposed by Shmuk (1): (1) When the picrates are formed, only the steam-volatile alkaloids rather than all the ether-extractable materials are present; (2) since the mixed picrate is recrystallized only once, the mother liquor instead of being recrystallized from hot water.

Table II. Effect of Methylation of Tobacco Alkaloids on Picrate Melting Point

Sample	Analysis		Nornicotine in Total Steam-Volatile Alkaloid %	Picrate Melting Point		
	Nicotine %	Nornicotine %		Original ° C.	Methylated ° C.	Mixed ° C.
Cash (blue-cured type)	0.70	2.40	77.4	182-185	221-223	220-223
Maryland Medium Broad-leaf (Robinson)	0.98	2.18	68.8	180-194	222-223	219-223
Burley, Halley	1.23	1.41	53.4	196-206	220-223	221-223
Maryland, Md.-Conn. Broadleaf	2.22	0.49	18.1	203-219	222-223	222-223
Xanthi (Turkish) <sup>b</sup>	6.59	1.23	16.3	214-223	221-224	221-224

<sup>a</sup> Not recrystallized.  
<sup>b</sup> American grown.

Melting points corrected and rounded off to whole numbers.

and again in the methylation process, the possibility of loss of the minor alkaloid is eliminated; and (3) the steam-distillation requires less time than an ether extraction. An improved steam-distillation apparatus (2) was used, but any steam-distillation apparatus may be conveniently used although the time may be longer and the volume of the distillate larger. When nicotine and nornicotine are determined (3), aliquants of the first distillate may be used to confirm the presence of nornicotine.

## SUMMARY

Nornicotine may be identified in tobacco, insecticidal tobacco preparations, and nicotine preparations by comparing the melting point of the mixed picrates of the steam-volatile alkaloids with the picrate melting point of a methylated sample thereof. Methylation of the nornicotine gives nicotine; consequently the picrate of the methylated alkaloids will melt at the same point as nicotine picrate and no depression of melting point will occur in a mixed-melting point determination with nicotine picrate in those cases where steam-volatile alkaloids other than nicotine and nornicotine are substantially absent.

Nornicotine was confirmed in tobacco samples by this method.

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# Colorimetric Determination of Phosphorus as Molybdivanadophosphoric Acid

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spectrophotometric study of the molybdivanadophosphoric acid method for the determination of phosphorus justifies its general recommendation for measuring this element colorimetrically. The experimental work covered the effects of the following variables:

YELLOW hue forms on adding an excess of a molybdate solution to an acidified solution mixture of a vanadate and orthophosphate. Presumably the colored component formed is molybdivanadophosphoric acid.

Mission (6) first proposed using this reaction as a basis for a colorimetric method for determining phosphorus in steel. Later Kovzov (1), Schröder (8), and Murray and Ashley (7) used it for steels, and Willard and Center (10) modified it for iron ores. Recently Koenig and Johnson (4) applied it to biological materials.

The general objective of the present work was to extend our knowledge of the analytical possibilities of this mixed heteropoly compound, since it is one of the few known examples of such compounds having direct value in colorimetry. More specifically, it seemed desirable to determine the general applicability of the procedure, weighing its merits relative to other available colorimetric methods for phosphorus, and to examine more critically its application to the determination of phosphorus in steel.

## GENERAL EXPERIMENTAL WORK

**APPARATUS AND SOLUTIONS.** Transmittancy measurements were made in 1,000- or 5,000-cm. cells with a General Electric recording spectrophotometer adjusted for a spectral band width of 10 m $\mu$ . If necessary to correct for a pale color in the reagents, a compensating blank was used in the reference beam of the spectrophotometer. Otherwise distilled water was used. A standard solution containing 0.1 mg. of phosphorus per ml. was prepared by dissolving twice recrystallized potassium hydrophosphate in water. A 5% solution of ammonium molybdate was prepared by dissolving the salt,  $(\text{NH}_4)_7\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$ , in warm water (50° C.). The vanadate solution was made by dissolving 5 grams of ammonium vanadate,  $\text{NH}_4\text{VO}_3$ , in 500 ml. of boiling water, cooling the solution somewhat, adding 20 ml. of concentrated nitric acid, and diluting to 1 liter after allowing the mixture to cool to room temperature. To determine the effect of various ions, nitrate, sulfate, or acetate salts were used for the standards, and sodium, potassium, or ammonium salts for the analyses. The various acids were analytical grade reagents.

**COLOR REACTION.** Although Mission (6) formulated the ammonium salt of the heteropoly complex as  $(\text{NH}_4)_3\text{PO}_4 \cdot \text{NH}_4\text{VO}_3 \cdot \text{MoO}_3$ , the exact nature of the compound is not clear. It cannot readily be fitted into either the Rosenheim or the Keggin formulas for heteropoly compounds. Presumably the acid is formed by substitution of both molybdenum and vanadium oxide radicals for oxygen in the phosphate radical to give a mixed heteropoly compound. If such is the case, the ratio of vanadium to phosphorus would have to be at least 2 to 1, and the ratio of molybdenum to phosphorus not greater than 10 to 1.

Application of the method of Vosburgh and Cooper (9) to determine these ratios was tried. Although previously this process has been used on relatively simple systems, it seemed that the ratio of vanadium to phosphorus might be determined by holding the molybdate and acid concentration constant. However, the results indicated that the ratio of vanadium to phosphorus is not constant. Attempts to determine the ratio of molybdenum to

acidity, reagent and phosphorus concentrations, temperature, order of adding reagents, stability, and some 60 diverse ions. As one result, an improved method is proposed for applying the method to determining phosphorus in plain carbon and low-alloy steels.

vanadium plus phosphorus failed, probably because most color reactions involving a heteropoly molybdate require a large excess of molybdate for color development. If one may reason from the results obtained, it appears that the compound is not of the type represented by the Rosenheim formulation  $\text{H}_7\text{P}(\text{Mo}_2\text{O}_7)_n(\text{V}_2\text{O}_6)_{6-n}$ . Although the constitution of molybdivanadophosphoric acid remains uncertain, fortunately the usefulness of the colorimetric procedure is unaffected.

**EFFECT OF VARIABLES ON THE COLOR DEVELOPMENT.** To study the effect of variables on the color development, the following experimental procedure was used:

A definite volume of phosphate solution, usually 5 ml., was placed in a 50-ml. volumetric flask, together with enough water to bring the volume of solution to about 20 ml. Five milliliters each of nitric acid (1 to 2), 0.25% ammonium vanadate solution, and 5% ammonium molybdate solution were added in order. Any precipitate formed was dissolved by mixing. The solution was then diluted to the mark with water and mixed, and the transmittancy curve determined.

**Acid Concentration.** In all previous work, except that of Willard and Center (10), nitric acid was used. If it is employed, there must be enough present to prevent the appearance of an orange-yellow color which forms in neutral or slightly acidic solutions. If the acidity exceeds 0.2 N, this color does not appear. Additional acid, up to 1.6 N, has no effect on the color, except that it develops more slowly at the higher acidities. At acidities above 1.6 N the color forms so slowly that a considerable negative error may arise. The optimum acidity of about 0.5 N is readily secured by using 5 ml. of nitric acid (1 to 2) per 50 ml. of final volume.

Sulfuric, perchloric, and hydrochloric acids behave much like nitric acid. If the acidity with any of them is less than 0.2 N, the orange-yellow hue develops in the blank. The desired color appears only slowly with 1 N hydrochloric acid, but development is complete within 5 minutes. Solutions 0.7 N in sulfuric acid, or 0.9 N in perchloric acid, behave similarly. At normalities of 1.4 and 1.7, respectively, full color is not developed in 5 minutes.

**Vanadate Concentration.** An excess of vanadate must be present for complete color development. Beyond this amount, additional reagent has no effect except for the slight color of the vanadate solution. For the present working conditions, 10 ml. ( $\pm 1$ ) of 0.25% reagent were satisfactory.

**Molybdate Concentration.** As in most procedures involving heteropoly compounds, excess ammonium molybdate must be present for complete color development. Beyond this amount, more reagent has no effect on the intensity of the color. Although prior recommendations specify a 10% solution, this has been changed to 5% because the solution is more easily prepared, the extra reagent is unnecessary, and the smaller concentration avoids the formation of the precipitate which may appear with use of the more concentrated solution.

**Order of Adding Reagents.** The reagents should be added in the order mentioned. If the acid follows the vanadate and



molybdate, the orange-yellow hue formed by these substances does not disappear readily, and a positive error results. If the molybdate is added to the acidified phosphate before the vanadate, yellow molybdiphosphoric acid is formed. If too much ammonium ion is present, a colloidal dispersion of ammonium molybdiphosphate may form. This precipitate does not disappear on adding vanadate. If the precipitate does not appear, the yellow solution is readily converted to molybdivanadophosphoric acid on adding vanadate.

**Temperature.** Most procedures specify adding the vanadate to a hot nitric acid solution, followed by cooling to room temperature before adding the molybdate. It makes no difference in the final results whether the solution is cooled to room temperature before or after adding vanadate, or after adding molybdate. A precipitate will appear, however, if the solution is boiled 15 to 20 minutes after adding molybdate.

**Phosphorus Concentration.** Transmittancy curves for solutions containing 1 to 100 p.p.m. of phosphorus are shown in Figure 1. Beer's law applies up to 40 p.p.m. for measurements made at 460  $m\mu$ .

**Stability of the Color.** The solutions used to determine the curves in Figure 1 were stored in Pyrex bottles and the transmittancies checked at measured time intervals for several weeks. Solutions containing 5 p.p.m., or more, are stable at least 7 weeks. Below this concentration, the color increases slowly, the error amounting to 2% in the transmittancy at 460  $m\mu$  in about 2 weeks.

**Diverse Ions.** To observe the effect of diverse ions 10 p.p.m. of phosphorus were used, the desired amount of diverse ion being included with the phosphorus solution. The apparent concentration of phosphorus was calculated from the transmittancy at 460  $m\mu$ . An error up to 2% was considered negligible.

The error does not exceed 2% for amounts up to 1000 p.p.m. of any of the following ions: aluminum, ammonium, barium, beryllium, cadmium, calcium, iron (III), lead, lithium, magnesium, manganese, mercury (I and II), potassium, silver, sodium,

strontium, tin (II), uranium, zinc, zirconium, acetate, arsenate, benzoate, bromide, carbonate, chlorate, citrate, cyanide, fluoride, iodate, lactate, molybdate, nitrate, nitrite, oxalate, perchlorate, periodate, pyrophosphate, salicylate, selenate, silicate, sulfate, sulfite, tartrate, and tetraborate.

Copper and nickel change the hue of the solution, and thus interfere with visual comparison; but up to 1000 p.p.m. of either of these metals does not interfere with spectrophotometric measurement at 460  $m\mu$ .

Only the ceric and tin (IV) ions precipitate under the conditions used.

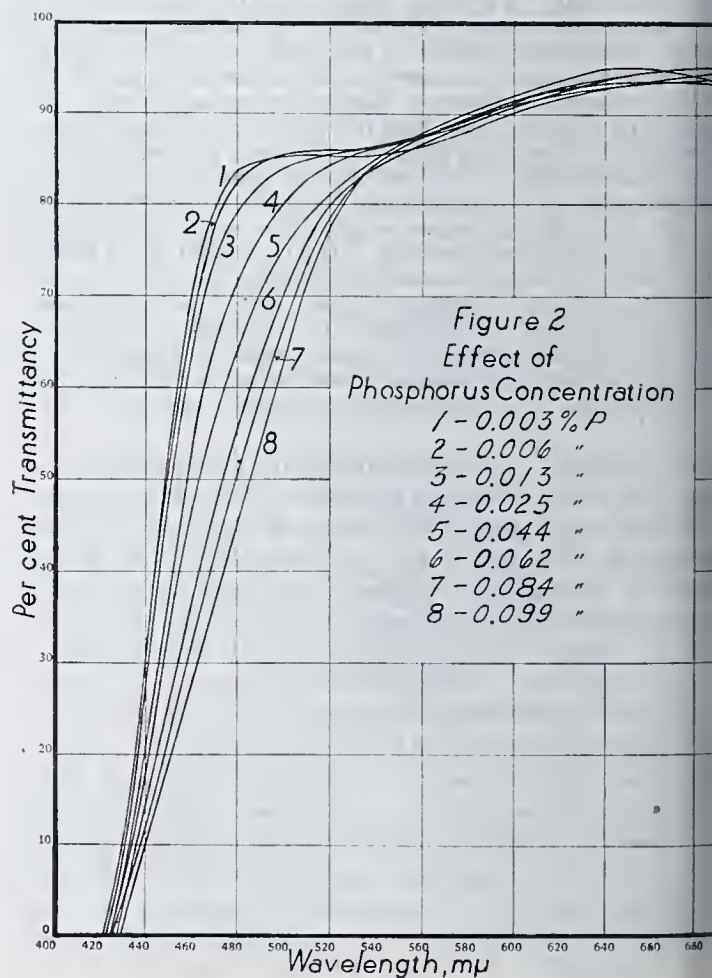
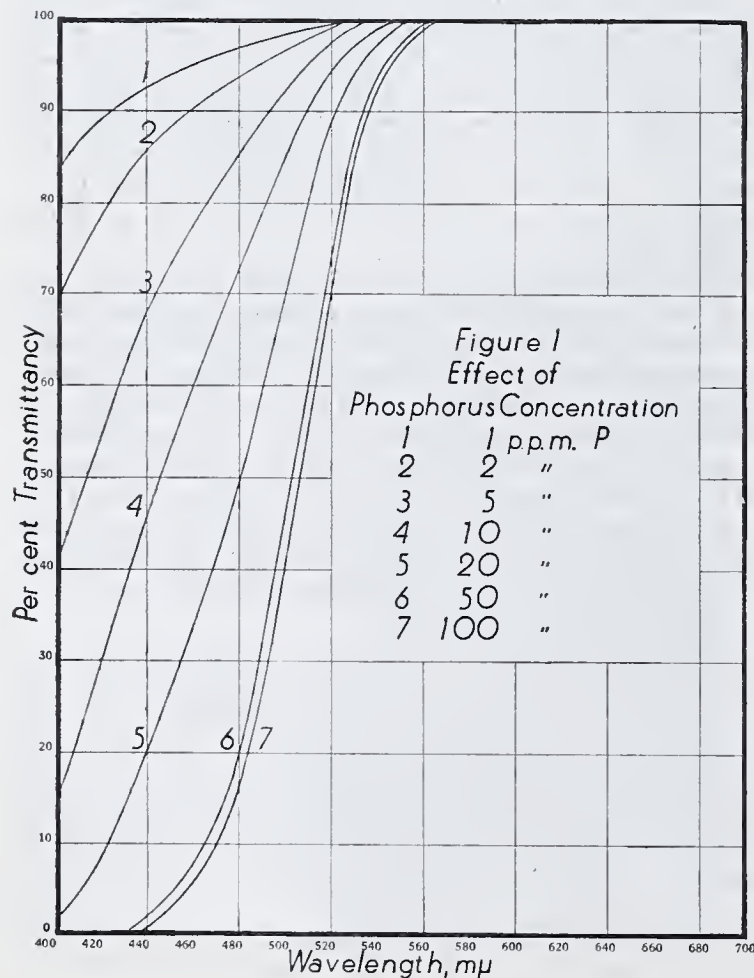
The largest single source of error is the effect of certain ions in slowing down the rate of the color reaction. Although this effect was noticed in a number of cases, generally the full color developed within 5 minutes. Bismuth, thorium, arsenate, chloride, fluoride caused considerable negative error, however, and full color did not develop in their presence in less than 30 minutes. The magnitude of such error may be reduced considerably by allowing the color to develop at least 30 minutes before measurement, or heating the solution in boiling water for 10 minutes after the final addition of reagent. But even then the error is negligible if 1000 p.p.m. of the ion are present. Heating is permissible only in the absence of silicates and arsenates, since they give a positive error under these conditions.

A few ions, such as iron (II), sulfide, thiosulfate, and thiocyanate, reduce the molybdivanadophosphoric acid or the excess molybdate to molybdenum blue.

The general effect of interfering diverse ions is summarized in Table I.

**DISCUSSION.** Although the molybdivanadophosphoric acid method has found little application for the determination of phosphorus, the procedure is rapid, sensitive, and relatively free from interference by most common diverse ions.

Comparison of this method with the various molybdenum blue procedures summarized by Woods and Mellon (11) shows that it possesses several advantages. The solutions are stable at least





weeks, as compared with a maximum of 10 hours for any of the molybdenum blues studied. It is also relatively free from interference, as compared with them. Especially important is this characteristic for iron (III) and silicate, both of which interfere in most molybdenum blue methods. Although a molybdenum blue more satisfactory for visual comparison than the yellow molybdenodiphosphoric acid, this technique can be used. However, photoelectric means are preferable. In general, the ranges of molybdenum blue methods are less than that for this procedure, which extends from 1 to 50 p.p.m. for 1-cm. thickness. The method conforms to Beer's law through most of this range. Since photoelectric measurement seems preferable, a series of permanent standards was not established. Such a series could be prepared from the acid itself, but with low concentrations of phosphorus the solutions are not stable more than 2 weeks. Comparison of the transmittancy curves in Figure 1 (for 10 p.p.m.) with those for dichromate solutions (3) shows an approximate match with the aqueous dichromate solutions at pH 6.1.

Table I. Effect of Diverse Ions

Added as	Amount P.p.m.	Error %	Amount Permissible P.p.m.
Bi(NO <sub>3</sub> ) <sub>3</sub>	1000	5	400
Cr <sub>2</sub> (SO <sub>4</sub> ) <sub>3</sub>	10	1	10
Co(NO <sub>3</sub> ) <sub>2</sub>	100	2	100
Fe(NH <sub>4</sub> ) <sub>2</sub> (SO <sub>4</sub> ) <sub>2</sub>	100	0	100
Th(NO <sub>3</sub> ) <sub>4</sub>	400	38	20
Na <sub>2</sub> HAsO <sub>4</sub>	1000	15	125
NaCl	1000	24	75
H <sub>2</sub> PtCl <sub>6</sub>	20	0	20
K <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub>	4	2	4
NaF	1000	51	50
KI	100	0	0
KMnO <sub>4</sub>	10	10	0
KSCN	500	0	500
Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub>	250	0	250
Na <sub>2</sub> WO <sub>4</sub>	250	2	250

oxidizes to iodine.

### RECOMMENDED GENERAL PROCEDURE

**SAMPLE.** Procure a representative portion of the material and subject it to the necessary preparative treatment. Weigh or measure by volume a sample containing not less than 0.005 mg. of phosphorus.

If necessary, dissolve the sample by appropriate means, taking care in the dissolution and subsequent treatment to convert the phosphorus to orthophosphate. Obviously, phosphoric acid should not be used for dissolution, nor pyrophosphate for a fusion. Interfering ions should either be removed or inhibited to bring their concentrations within the limits set in Table I. Make the solution just acidic to litmus and dilute to a definite volume in a volumetric flask.

**DESIRED CONSTITUENT.** Transfer a suitable aliquot of the solution to a 100-ml. flask. If Nessler tubes are used for visual comparison, at least 0.005 mg. of phosphorus should be in the aliquot. For photometric measurement in 1-cm. cells, 0.1 to 5 μg. of phosphorus should be present. Add in order, with adequate mixing, 10 ml. of nitric acid (1 to 2), 10 ml. of 0.25% ammonium vanadate solution, and 10 ml. of 5% ammonium molybdate solution. Dilute to the mark and mix well. The color may be measured at once by any of the usual means. Filter photometric measurement should be made with a blue filter having its maximum transmission near 470 mμ, and spectrophotometric measurement is made best between 460 and 480 mμ.

### DETERMINATION OF PHOSPHORUS IN STEEL

Several colorimetric methods have been described for determining phosphorus in steel in a fraction of the time required for the conventional gravimetric or titrimetric procedure. A 0.5-gram sample may be handled colorimetrically with an accuracy equal to that of the usual titrimetric procedure, and with a saving of at least two thirds. For this purpose Mission (6) first used the molybdivanadophosphoric acid method. It was later adapted subsequently by others (1, 7, 8). Since the procedure

has not been generally applied in the routine analysis of steel, the work reported here was done to check the earlier recommendations with modern photoelectric equipment.

### EXPERIMENTAL WORK

A plain carbon steel was used as the source of phosphorus in most of the work. The manufacturer's analysis gave the following percentages: C, 0.64; Mn, 0.65; Si, 0.18; S, 0.022; and P, 0.02. The last value was checked. The solutions of ammonium vanadate and molybdate were the same as used before. The potassium permanganate and the ammonium peroxydisulfate solutions were 1 and 7.5%, respectively. The 3% hydrogen peroxide was the common U.S.P. solution.

**PRELIMINARY EXPERIMENTS.** In most methods for the colorimetric determination of minor constituents in iron and steel, some reaction is used to convert the colored ferric ion to a colorless complex. Several common reactions are inapplicable with the molybdivanadophosphoric acid method. Phosphoric acid obviously cannot be used to form the ferric phosphate complex. Fluoride ion interferes with the color development, thus eliminating the fluoride complex. Reduction of ferric to ferrous ion is objectionable because ferrous iron partially reduces the colored complex. Although dissolution of the sample in perchloric acid gives a measurable system (10), the procedure recommended here proved to be more rapid and more easily controlled.

This situation necessitates developing the molybdivanadophosphoric acid color in the presence of the ferric iron color. The latter, or background color, must be reproducible to ensure reliable results in the final evaluation of the total color. Preliminary experiments with the procedure of Murray and Ashley (7) revealed difficulties which were traced to this background color. The deviation was small and probably would be unnoticed in various visual methods. Since it was definitely more than the 2% error considered negligible for work with the recording spectrophotometer, means were sought to reduce the uncertainty.

Subsequent work showed that a reproducible background color could be obtained with the procedure of Murray and Ashley only by careful control of the experimental conditions. The use of ammonium peroxydisulfate as oxidant eased the necessity for careful control. Solutions prepared with this reagent were more reproducible than those using the permanganate previously recommended, and they did not require such close duplication of experimental conditions. Excess oxidant is readily removed by heating, thus eliminating an operation from the procedure and saving time.

**EFFECT OF VARIABLES ON COLOR.** The following experimental procedure was used in studying the effect of variables on the peroxydisulfate method:

A 0.5-gram sample of steel in a 150-ml. conical flask was treated with 20 ml. of nitric acid (1 to 2). After violent action ceased, the solution was heated to boiling on a hot plate and allowed to boil from 2 to 5 minutes. Five milliliters of the peroxydisulfate solution were added, and the solution was boiled from 3 to 5 minutes to destroy excess oxidant. Ten milliliters of vanadate solution were then added, and the solution was cooled to room temperature. Following addition of 20 ml. of molybdate solution, the system was mixed and transferred to a 100-ml. volumetric flask. After dilution to volume with water and mixing, the transmittancy curve was determined in 5.00-cm. cells.

**Acid Concentration.** The concentration of acid in the final solution is indefinite because of the amount used up in the dissolution process, and that boiled out during subsequent heating. The intense color produced with the use of small amounts of acid is probably attributable to a vanadomolybdate complex which forms at low acidity. The color with high acid concentration is due almost entirely to the iron color. The optimum amount of acid is 20 ml. of nitric acid (1 to 2). This volume provides reproducible background color, but more reduces the intensity of the final color. Measurement of the acid to ±1 ml. from a 250-ml. buret is recommended.



**Peroxydisulfate Concentration.** Enough ammonium peroxydisulfate must be added to oxidize organic material, but a large excess should be avoided. Although 5 ml. of a 7.5% solution were chosen as the optimum amount, twice as much is permissible. As the solution decomposes on standing, it should be prepared daily.

**Vanadate and Molybdate Concentration.** Variations in the vanadate and molybdate concentrations have some effect on the final color. Twenty milliliters of 5% ammonium molybdate and 10 ml. of 0.25% ammonium vanadate gave best results. These volumes should be controlled within 1 ml.

**Time of Heating.** The steel solution should be boiled at least 2 minutes after dissolution of the sample, but boiling as long as 5 minutes has no deleterious effect on the color. The length of time of boiling after adding peroxydisulfate is apparently not critical, since variation from 3 to 10 minutes made no difference.

**Order of Adding Reagents.** Three successions of adding reagents were tried: both the vanadate and the molybdate to the hot steel solution; the vanadate to the hot solution, followed by cooling before adding the molybdate; and both the molybdate and vanadate to the cooled steel solution. The same final color was obtained. However, the second order was finally adopted, chiefly because of previous recommendation.

**Stability of Color.** The full color intensity develops immediately on adding the molybdate, and the color is stable for at least an hour. Since the method is being recommended for rapid photometric measurement, this factor was not studied further.

### RECOMMENDED PROCEDURE FOR STEELS

Based on this experimental evidence, the following procedure is recommended for the determination of phosphorus in plain carbon and certain low alloy steels:

**SAMPLE.** Weigh a 0.5-gram representative sample into a 150-ml. conical flask, add 20 ml. of nitric acid (1 to 2), and, after violent action ceases, boil 2 minutes to remove nitrous oxide fumes. Add 5 ml. of a freshly prepared 7.5% solution of ammonium peroxydisulfate, and boil 3 to 5 minutes to destroy the excess.

**DESIRED CONSTITUENT.** Add 10 ml. of 0.25% ammonium vanadate solution to the hot solution and then cool to room temperature. After adding 20 ml. of 5% ammonium molybdate solution, mix thoroughly and transfer the solution to a 100-ml. volumetric flask. Dilute to the mark, mix, and measure the color intensity by any suitable means. A blue filter with maximum transmission near 470 mμ is suitable for a filter photometer. With a spectrophotometer the best wave length is the range 460 to 480 mμ.

Table II. Data Used for Calibration Curve

Steel No.	Phosphorus %	$T_{460}$ %	Log $T_{460}$	
			Observed	Calcd. <sup>a</sup>
55	0.003	68.8	1.838	1.843
11d	0.006	68.0	1.833	1.830
12d	0.013	62.9	1.799	1.798
16b	0.025	55.5	1.744	1.744
20c	0.044	45.9	1.662	1.658
21c	0.062	36.8	1.566	1.576
22b	0.084	31.2	1.494	1.476
8d	0.099	25.0	1.398	1.409

<sup>a</sup> Calculated from the equation

$$\% P = \frac{1.857 - \log T}{4.52}$$

### ANALYSIS OF STANDARD SAMPLES

The final test of the procedure was the analysis of some 20 plain and low-alloy steels from the National Bureau of Standards. Eight plain carbon steels of different phosphorus contents were used to establish a calibration curve. The remainder were analyzed on the basis of this curve. Three samples of each steel were

prepared according to the recommended procedure. The average of the transmittancy readings at 460 mμ was used as the probable value.

The data secured for the eight steels used for the calibration curve are summarized in Table II. The transmittancy curves for the solutions are shown in Figure 2. If log  $T_{460}$  is plotted against percentage of phosphorus, a straight line is obtained indicating conformity to Beer's law. The equation of the straight line was calculated by the method of least squares, data obtained being included in Table II.

After the calibration curve had been established, 14 plain carbon and low-alloy steels were analyzed (Table III). In each case the average value found is the result of at least 3 determinations. The average deviation from the mean was usually less than 3% of the phosphorus present.

Table III. Analysis of Standard Steels

Sample Type and No.		Phosphorus Found %	Deviation from Standard %	Allowable Deviation %
Bessemer	9c	0.091	-0.005	±0.005
Bessemer	10d	0.087	0.001	0.005
Bessemer	23a	0.102	0.000	0.005
B.O.H.	13c	0.011	-0.002	0.005
B.O.H.	15a	0.006	-0.001	0.005
A.O.H.	21a	0.062	-0.001	0.005
A.O.H.	34	0.095	0.000	0.005
A.O.H.	35	0.035	+0.002	0.005
Electric	51	0.016	+0.005	0.005
Acid electric	65a	0.036	0.000	0.005
Mn rail	100	0.022	-0.001	0.005
Ni	33b	0.036	-0.001	0.005
Ni-Cr	32b	0.024	+0.008	0.005
Cr-Mo	72	0.033	+0.017	0.005

Examination of the results shows that in four cases the experimental results are outside the limits set for gravimetric or photometric methods. The reason for the deviation in steel 9c is unknown, three excellent checks being obtained. With steel 100, however (because of high carbon?), 6 samples diverged widely. Other samples in which deviation occurred contained moderate amounts of chromium, which would be expected to interfere.

The accuracy of the calibration curve was confirmed by calculating the best straight line for all the steels except Nos. 33b, 51, and 72. The formulas for the best straight lines are

$$\begin{aligned}\% P &= \frac{1.857 - \log T_{460}}{4.529} \quad (8 \text{ samples}) \\ &= \frac{1.857 - \log T_{460}}{4.500} \quad (18 \text{ samples})\end{aligned}$$

**DISCUSSION.** The use of ammonium peroxydisulfate has several advantages over potassium permanganate in this method. The background color is more nearly reproducible. Since control of experimental conditions is not necessary, the overall procedure is simplified. There is a saving of approximately 10 minutes in time.

The calibration curve may be prepared by using a series of steels of known phosphorus content, or by adding known amounts of phosphorus to solutions of a single analyzed steel. The former alternative was used in this work, and the latter by Murray and Ashley (?). Their method seems preferable if one type of steel is to be analyzed, since the background color is then essentially constant. As Beer's law applies to the system, one may extrapolate to concentrations of phosphorus below that of the standard. If several types of steel, having compositions not too different, giving different background colors, are to be analyzed, an independent calibration curve should be prepared from a series of known steels of the types to be analyzed. In any questionable case the second alternative should be used. Instead of taking the results from such a calibration curve, they may be calculated from the straight-line equation already mentioned.



Murray and Ashley reported serious interference with high (%) silicon, but in the usual range of this element no interference as found in this work. More than 0.4% chromium gives a positive error. In general, the accuracy equals that of the common gravimetric method, which is less rapid and requires more skill. The molybdenum blue method of Hague and Bright (2) is also as rapid.

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ABSTRACTED from a portion of the thesis presented by R. E. Kitson to the Graduate School of Purdue University in partial fulfillment of the requirements for the degree of doctor of philosophy.

## Determination of Potassium in Fertilizer Mixtures Removal of Ammonia and Organic Matter without Ignition

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THE ignition step in the A.O.A.C. (1) method for the determination of potassium in mixed fertilizers requires careful attention and may sometimes result in potassium losses from insoluble residues. This paper presents a procedure which has been found to reduce errors and shorten the time of analysis on many fertilizers by eliminating high-temperature ignition with sulfuric acid.

Kraybill and Thornton (4) have called attention to losses from ignition which may be caused by spattering or volatilization. St. John and Midgley (6) found that controlled temperatures tend to avoid volatilization of potassium due to localized overheating. They also noted insoluble residues from ashing of materials with sulfuric acid, but usually none when using their acid digestion method.

In his 1940 Report on Potash, Ford (3) showed that errors from "water-insoluble residues that are often encountered" could be corrected either by filtering the potassium solution before adding platinic chloride, or by dissolving the weighed potassium platinic chloride with hot water and reweighing the dried crucible. In either case additional work is involved which increases the time of analysis.

In 1934 this laboratory experienced difficulty in obtaining accurate potash results by the official method of that time when analyzing fertilizers containing little or no superphosphate and large amounts of monoammonium phosphate. Since the ignition step was found to be the main source of error in the analysis of mixtures of this type (2), a procedure was adopted which eliminated ignition by using a low-temperature method to remove interfering ammonium salts and organic matter. Thus it was possible to form insoluble metasilicates or phosphates.

Changes in the A.O.A.C. method were made in 1935, so that with proper manipulation and corrections for insoluble residue was possible to determine potassium in all types of fertilizer with a greater degree of accuracy. Although these changes included better ignition technique, the low-temperature procedure used in this laboratory appeared to have certain advantages. Several improvements in the method have been made recently and analytical results compared with those obtained by ignition with sulfuric acid, using the same solution for both determinations.

#### METHOD

REAGENTS (other than used in A.O.A.C. method). Concentrated sodium hydroxide solution, 30 grams of A.C.S. reagent sodium hydroxide per 100 ml. of solution. Sodium chlorate solution, 10 grams of A.C.S. reagent sodium chlorate per 100 ml. of solution. 30% sulfuric acid, 20 ml. of sulfuric acid (A.C.S. reagent, 1.84 sp. gr.) in 80 ml. of water.

METHOD 1 (for inorganic fertilizers). Place 5 grams of sample in a 250-ml. volumetric flask and add about 100 ml. of water and 50 ml. of saturated ammonium oxalate solution. Boil 30 minutes, cool, dilute to 250 ml., mix well, and filter or allow coarser particles to settle. Pipet a 100-ml. aliquot into a 500-ml. Kjeldahl flask containing a few glass beads. (If moisture content of the fertilizer permits grinding fine enough to prevent separation of particles, weigh a 2-gram sample directly into the Kjeldahl flask. Add about 75 ml. of water and 20 ml. of saturated ammonium oxalate solution. Boil 10 minutes.)

Add 3 or 4 drops of 1% phenolphthalein solution and 5 ml. of concentrated sodium hydroxide solution. Boil vigorously until damp red litmus paper placed over mouth of flask shows no trace of blue color when left there several minutes. The time required to expel ammonia is usually less than 15 minutes. If red color of phenolphthalein fades during boiling, add sufficient sodium hydroxide to restore.

Cool and transfer with thorough washing to a 200-ml. volumetric flask. Dilute to mark, mix well, and pass through a close-textured dry filter. Determine  $K_2O$  on a 25- or 50-ml. aliquot by the platinic chloride method. Treat precipitate in evaporating dish with about 15 ml. of 85% alcohol and 1 ml. of concentrated hydrochloric acid. Use rubber policeman to break up residue thoroughly before transferring to Gooch crucible. Wash as usual with alcohol and ammonium chloride solution, but use ten 10-ml. portions of ammonium chloride solution if a 50-ml. aliquot was taken. Adjust suction during the ammonium chloride washing so that the jet from wash bottle or 10-ml. pipet (enlarged tip opening) will about half fill the crucible and agitate the precipitate with each washing.

METHOD 2 (for fertilizers containing urea, cyanamide, or other interfering organic materials). Prepare a 5-gram to 250-ml. fertilizer solution as in Method 1, but after boiling 30 minutes add 2 ml. of concentrated ammonium hydroxide or sufficient to make alkaline. Cool, dilute to volume, mix well, and pass through a close-textured dry filter. Add 5 ml. of concentrated nitric acid to a 100-ml. aliquot in a 500-ml. Kjeldahl flask and boil vigorously to a volume of about 10 ml. Add 5 ml. of sodium chlorate solution and 5 ml. of 30% sulfuric acid. (If sample is high in organic matter or urea, use 10 ml. of 30% sulfuric acid.)

Boil to dense sulfur trioxide fumes using low heat, then use high heat for 10 to 15 minutes to destroy organic matter. Cool 3 or 4 minutes and wash down neck of flask with about 100 ml. of water. Heat to obtain complete solution of residue. Add phenolphthalein and neutralize with concentrated sodium hydroxide solution. Add 2 ml. excess and boil to negative test for ammonia. Proceed as in Method 1.

#### DISCUSSION

The use of a 5-gram sample in this method rather than the official 2.5 grams is based upon the study of Kraybill and Thornton (5), who found that errors may result due to difficulty in weighing uniform 2.5-gram samples. The larger sample is taken in order to minimize such errors, usually caused by separation



Table I. Comparative K<sub>2</sub>O Analyses and Composition of Laboratory Mixtures

Mixture <sup>a</sup>	Calculated K <sub>2</sub> O %	H <sub>2</sub> SO <sub>4</sub> Ignition K <sub>2</sub> O %	New Method K <sub>2</sub> O %	Composition of Mixture %
L-1	22.2	22.14 22.32	22.19 22.23	20 KCl <sup>b</sup> 20 K <sub>2</sub> SO <sub>4</sub> <sup>c</sup> 10 Superphosphate 25 (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> 20 Ammo-Phos A 5 Bone meal
	Av.	22.23	22.21	
L-2	22.2	21.93 <sup>d</sup> 22.24	22.17 22.10	20 KCl <sup>b</sup> 20 K <sub>2</sub> SO <sub>4</sub> <sup>c</sup> 10 Superphosphate 10 Ammo-Phos A 10 CaCN <sub>2</sub> 10 Uramon 10 Fish meal 10 Bone meal
	Av.	22.09	22.14	
L-3	30.4	30.28 30.44	30.35 30.30	50 KCl <sup>e</sup> 10 Superphosphate 20 (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> 10 Ammo-Phos A 10 Tankage
	Av.	30.36	30.33	
L-4	30.4	30.37 30.43	30.42 30.36	50 KCl <sup>e</sup> 10 Superphosphate 20 (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> 10 Ammo-Phos A 10 Uramon
	Av.	30.40	30.39	
L-5	30.4	30.18 30.40	30.41 30.29	50 KCl <sup>e</sup> 20 Superphosphate 20 (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> 10 Ammo-Phos A
	Av.	30.29	30.35	
L-6	10.8	10.75	10.76	20 K <sub>2</sub> SO <sub>4</sub> C.P. <sup>f</sup> 40 NaNO <sub>3</sub> 40 (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>

<sup>a</sup> Materials weighed on analytical balance. Duplicate analyses made on a second series of weighings.

<sup>b</sup> Trona muriate. Analysis by this laboratory, 61.29% K<sub>2</sub>O. Manufacturer's analysis, 61.26% K<sub>2</sub>O.

<sup>c</sup> Foreign potassium sulfate. Analysis by this laboratory, 49.70% K<sub>2</sub>O. Manufacturer's guarantee, 49.5% K<sub>2</sub>O.

<sup>d</sup> Low result probably caused by spattering at start of ignition due to large amount of organic matter.

<sup>e</sup> Trona muriate. Analysis by this laboratory, 60.80% K<sub>2</sub>O. Manufacturer's analysis, 60.81% K<sub>2</sub>O.

<sup>f</sup> Analytical reagent, dried before using. Theoretical K<sub>2</sub>O, 54.05%.

of coarse 1-mm. particles from the fines. Many fertilizers of low moisture content, however, can be finely ground and have only traces of organic matter from phosphates and by-product ammonium sulfate. Two grams of such material may be treated directly for ammonia removal by the shortened procedure. The total time from weighing a 2-gram sample to taking an aliquot for evaporation with platinic chloride is about 40 minutes.

The time of boiling a 5-gram sample with ammonium oxalate has been made to agree with the A.O.A.C. period of 30 minutes, but it has been the experience of this laboratory that 10 to 15 minutes is sufficient for most types of fertilizer, especially when the composition of the sample is known or a finely ground 2-gram sample is used.

Although Method 1 does not require addition of ammonium hydroxide after boiling with ammonium oxalate, this may be done if the solution is to be used for a check analysis by sulfuric acid ignition. This does not appreciably lengthen the time of expelling ammonia from a 100-ml. aliquot.

Phenolphthalein indicator is not destroyed or volatilized during the boiling period. Fading of color indicates a drop in pH and will occur only in exceptional cases where 5 ml. (1.5 grams) of sodium hydroxide are not sufficient to react with all ammonium salts present. If it is desired to check the litmus test for complete removal of ammonia, phenolphthalein may be omitted and a portion of the final filtered solution treated with Nessler reagent.

The final solution of a fertilizer analyzed by Method 1 will contain a small amount of sodium oxalate. In case the sample contains no soluble calcium the amount of sodium oxalate in a 50-ml. aliquot taken for evaporation with platinic chloride will be approximately 0.24 gram. This may be decomposed before precipitation of potassium platinic chloride by the hypochlorite reaction, or preferably allowed to remain with the precipitate,

since this amount of sodium oxalate is easily removed by an alcohol and ammonium chloride washing. Decomposition of sodium hypochlorite is accomplished by the addition of 3 to 5 ml. of pure 5% available chlorine solution to a 50-ml. aliquot in a porcelain dish. The solution is then made distinctly acid with concentrated hydrochloric (2 ml.) and evaporated for 10 minutes, after which the necessary amount of platinic chloride is added.

The final solutions of organic fertilizers will not contain oxalate since it is destroyed by the sulfuric acid treatment of Method 1. In this procedure sodium chlorate is used in addition to sulfuric acid in order to destroy other forms of organic matter, especially cyanamide, urea, tankage, and fish meal were used in amounts when present in large amounts. No attempt was made to use perchloric acid because of the possible hazard of explosion.

Table I gives the comparative analyses and composition of six fertilizer mixtures made in the laboratory, each containing known amounts of K<sub>2</sub>O. Interfering materials such as calcium cyanamide, urea, tankage, and fish meal were used in amounts greater than those ordinarily found in commercial fertilizers. One inorganic mixture (L-6) which contained no calcium was included, so that the maximum amount of sodium oxalate would be present.

In these particular mixes, with the exception of L-1, ignited with sulfuric acid according to the A.O.A.C. technique produced only traces of insoluble residue. The asbestos-padded Gooch crucibles were therefore not washed out with hot water and weighed. Table II gives comparative analyses on various types of local commercial fertilizers.

Table II. Comparative K<sub>2</sub>O Analyses of Commercial Fertilizers

Formula	H <sub>2</sub> SO <sub>4</sub> Ignition K <sub>2</sub> O %	New Method K <sub>2</sub> O %	Variation K <sub>2</sub> O %
7-20.5-17	18.07	18.02	-0.05
7-20.5-17 <sup>a</sup>	16.48	16.49	+0.01
7-20.5-17 <sup>b</sup>	17.86	17.80	-0.06
6-20-12	13.32	13.31	-0.01
8-12.5-6	5.72	5.77	+0.05
7-3.5-23	23.01	23.15	+0.14
6-15-10	9.98	9.91	-0.07
7-10-10	10.17	10.23	+0.06
7-11-10	10.48	10.50	+0.02
8-9-11	10.68	10.59	-0.09
7-12-7	7.83	7.79	-0.04
11-20-22	26.63	26.61	-0.02

<sup>a</sup> Containing 252 pounds of bone meal per ton.

<sup>b</sup> Containing 50 pounds of Uramon per ton.

## SUMMARY

A method presented for the determination of potassium in mixed fertilizers involves the usual ammonium oxalate solution and chloroplatinic acid precipitation, but employs a rapid volatilization procedure for removal of ammonia, and an oxidizing method for destroying interfering organic compounds. The means of preparing a solution from which potassium may be precipitated makes it unnecessary to ignite at a high temperature with sulfuric acid, which may result in the formation of insoluble residue or the loss of potassium by spattering or volatilization.

A large number of analyses by this method have been made on inorganic and organic fertilizers containing salts of known potassium content, and the results are in close agreement with the calculated values of these mixtures.

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# Relationship between Unsaturation and the Ultraviolet Absorption Spectra of Various Fats and Fatty Acids

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The spectral absorption of several unsaturated fatty acids and natural fats have been measured from 2500 to 2100 Ångstrom units. The curves are presented that show a definite relationship between the degree of unsaturation and extinction coefficients at 2100 Ångstrom units. From the composition of natural fats it is possible to predict the extent of absorption at this wave length.

It is well known that absorption by the carbon to carbon double bond, one of the most important chromophores (3, 6), is modified by factors such as the cis- and trans-configuration, the lighting by substituent groups, the number of double bonds in the carbon chain, and their positions relative to each other (3). The natural fatty acids, their esters, and isomers constitute a most important group of aliphatic compounds differing chiefly in the number and position of double bonds. In a recent review (2) it was pointed out that except for the saturated fatty acids (13) the absorption curves of the well-known members of this series of compounds and the natural oils have usually not been extended below 2200 or 2300 Å. (1, 4, 5, 7, 9, 14, 19). The measurements that have been made in the longer wave lengths show such irregularities that it must be assumed that impurities with strong absorption bands are affecting the results.

The effect of increasing numbers of unconjugated double bonds in simple hydrocarbons is so marked at 2100 Å. (3, 6) that it was decided to study the absorption spectra of the highly purified fatty acids. Although it was not possible to extend the curves of unsaturated acids to their maxima (below 2000 Å.) because of the limit of the spectrograph (2100 Å.), there nevertheless was found a large and consistent effect of increasing unsaturation which seemed of practical importance, since this wave length is within the range of many spectrographs now in use.

## EXPERIMENTAL

The absorption measurements from 2100 to 2250 Å. were made with a Gaertner Littrow spectrograph. From 2300 to 2500 Å. absorption was measured with a photoelectric spectrophotometer similar to that described by Hogness *et al.* (8). The solvent employed was purified ethyl alcohol (commercial 95% alcohol freshly distilled from potassium hydroxide) for all samples except stearic acid which was dissolved in ethyl ether (freshly opened anesthesia grade). The absorption values of the compounds are plotted as the logarithm of the molecular extinction coefficients,  $\epsilon$ , while the values for the oils are expressed as 1%, where 1% means 1 gram in 100 cc. of solution. The curves were calculated from Lambert's and Beer's law,

$$\log \frac{I_0}{I} = \epsilon cl$$

Measurements were made on samples of the highest purity obtainable. (The authors are indebted to J. P. Kass and J. Nichols for the preparation of these materials.) The stearic acid melted at 9.6° in a capillary tube and had no measurable iodine number. Oleic acid was prepared by repeated recrystallization at low temperature of the fatty acids of olive oil until a sample with iodine number (Wijs) of 88 was obtained. The chief impurity probably was palmitic acid. The methyl esters of linoleic, linolenic, and arachidonic acids were made from the recrystallized polybromides and debromination in methyl alcohol. The iodine number of each preparation was within 2 units of the theoretical value.

The curves in Figure 1 show the marked effect of unsaturation on spectral absorption below 2250 Å. At 2100 Å. the long-chain fatty acids have the following molecular extinction coefficients: stearic 60; oleic 180; methyl linolate 2500; methyl linolenate 10,000; and methyl arachidonate 14,500. In other words, arachidonic acid with an iodine number approximately 4 times that of oleic acid has a spectral absorption at 2100 Å. which is roughly 80 times as great.

The suggestion of an absorption band at 2350 Å. may be due to trace impurities. This is the region of maximum absorption by conjugated dienes and it is known that in the saponification, bromination, debromination, and distillation of highly unsaturated fatty acids some conjugation may take place (16, 17, 18). However, since the conjugated dienes have molecular extinction coefficients of 20,000 to 30,000 in this region (2), there could not be more than a fraction of 1% present in any of these preparations. This would not measurably affect the absorption values of the highly unsaturated acids at 2100 Å. although it is sufficient to throw the curves out of line at 2300 Å.

The large and regular effect of unsaturation on light absorption at 2100 Å. is contrasted with the smaller and irregular effects at the longer wave lengths in Figure 2. It is clear that if absorption at 2100 Å. can be measured with sufficient accuracy the values can be used as constants in simultaneous equations for calculating the fatty acid composition of oils. This direct measurement may well be used instead of the one described by Kass *et al.* (12) and later extended by Mitchell *et al.* (15), which depends upon the measurement of the conjugated linoleic and linolenic acids after saponification at a high temperature. The chief disadvantage of the present method comes from the requirement that the measurements be made at a wave length shorter than that reached by many instruments.

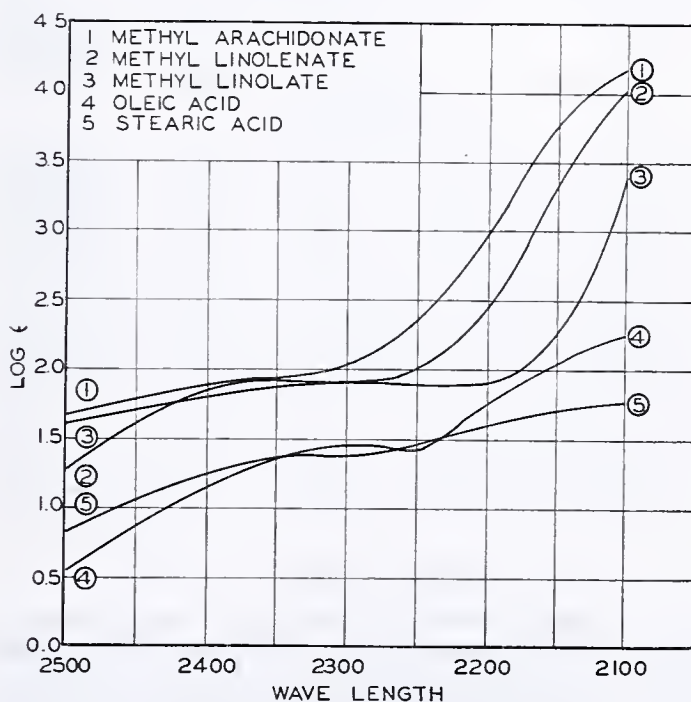


Figure 1. Absorption Spectra of Five Fatty Acids with Different Numbers of Unconjugated Double Bonds



Table I. Comparison of Calculated and Experimentally Determined Extinction Coefficients for Natural Fats

Fat	Iodine Value (Wijs)	Thiocyanogen Value	Saturated Acids	Oleic Acid	Linoleic Acid	Extinction Coefficients,	
						$E_{1\text{ cm. at } 2100 \text{ Å.}}^{1\%}$ Calculated	Measured
Coconut	8.6	7.3	87.4	6.6	1.5	3.8	5.9
Olive	83.2	74.8	11.8	74.0	9.7	15.2	18.2
Corn (Mazola)	134.2	82.8	6.5	29.6	59.4	61.6	60.0

In Figure 3 the curves of four plant fats of widely different composition are compared with those of the fatty acids. The extinction coefficients,  $E_{1\text{ cm.}}^{1\%}$ , at 2100 Å. are in the range that would be expected from the composition of the fats. Iodine numbers (Wijs) and thiocyanogen numbers were determined for coconut oil, olive oil, and corn oil. (The authors are indebted to H. G. Loeb for these determinations.) From these analytical constants the composition of the three fats was calculated (Table I). The corrected value for the thiocyanogen number of linoleic acid as given by Kass *et al.* (11) was substituted in Equation 3 of the following simultaneous equations described by Jamieson (10):

$$x + y + s = 1 \quad (1)$$

$$86.01x + 173.20y + o = I. N. \quad (2)$$

$$86.01x + 90.59y + o = T. N. \quad (3)$$

where  $x$  is the amount of oleic acid glyceride;  $y$ , the linoleic acid glyceride; and  $s$ , the saturated acid glyceride present in the fat. After determining the composition of the fat, and converting to the free acids (95.5% of the glycerides) the values were substituted in the equation:

$$7.1x + 100y + 2.1s = E_{1\text{ cm.}}^{1\%}$$

The  $E_{1\text{ cm.}}^{1\%}$  at 2100 Å. for oleic acid is 7.1; for linoleic acid, 100;

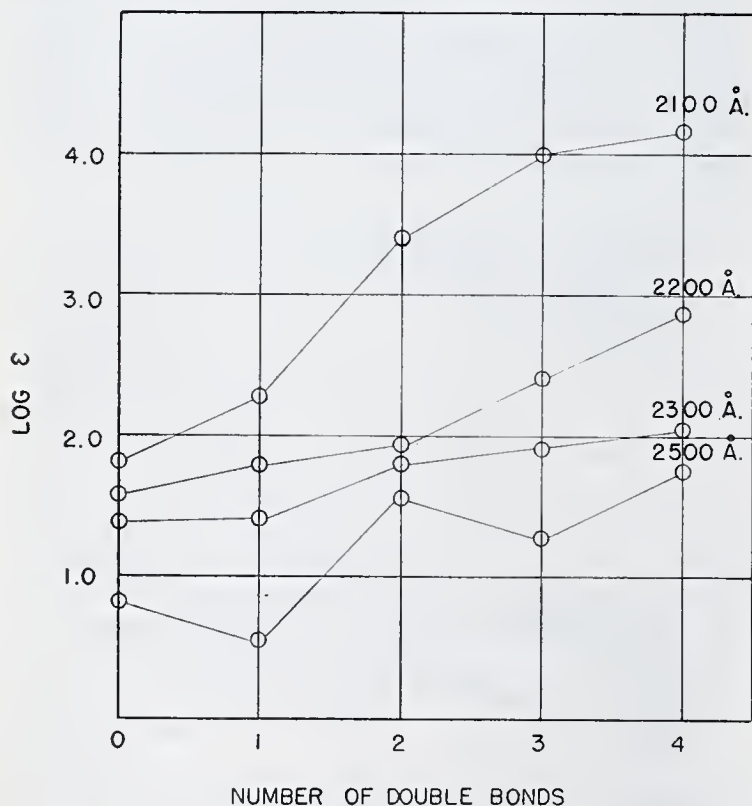


Figure 2. Effect of Number of Unconjugated Double Bonds in Fatty Acids on Extinction Coefficient at Different Wave Lengths

Experimental points are connected by lines to aid in following values for same wave length. Mixtures of two fatty acids differing by one double bond would give intermediate values on straight line between them but an oil averaging one double bond by having an equal amount of saturated acid and linoleic acid would not have absorption of oleic acid glyceride (1 double bond)

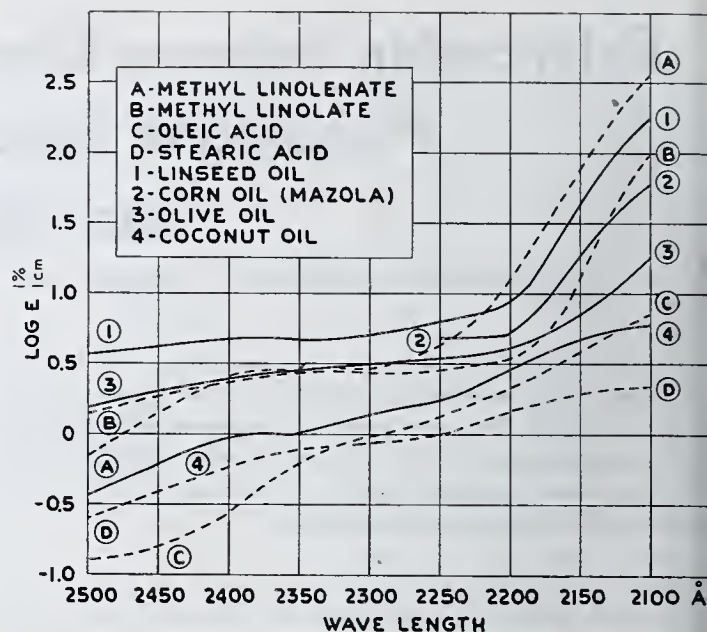


Figure 3. Absorption Spectra of Four Vegetable Oils Compared with Their Constituent Fatty Acids

and for stearic acid, 2.1. The  $E_{1\text{ cm.}}^{1\%}$  calculated in this manner were then compared with the values determined experimentally. The results (Table I) are seen to be of the right order of magnitude. Both coconut oil and olive oil have very low spectral absorption at 2100 Å. and are thus subject to considerable error introduced by traces of highly absorbing materials. A small error in the calculation of the linoleic acid content would also have a large effect. For example, if the coconut oil really contained 2.5% linoleic acid instead of the calculated 1.5%, the  $E_{1\text{ cm.}}^{1\%}$  would be raised to 5.6. However, corn oil absorption is of such magnitude that the effect of contamination is minimized and, consequently, it is possible to show a close agreement between the calculated and experimental values for this fat.

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# 8-Hydroxyquinaldine as an Analytical Reagent

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8-Hydroxyquinaldine is a more selective reagent than 8-hydroxyquinoline because it does not precipitate aluminum. Separations of zinc from magnesium, from aluminum, and from magnesium and aluminum are given. The precipitates of the zinc and the magnesium complex salts may be either weighed or determined volumetrically by bromination. Aluminum may be determined in the filtrate of the zinc determination by adding 8-hydroxyquinoline. The effect of the pH upon the completeness of precipitation of the 8-hydroxyquinaldine complexes of cupric, zinc, ferric, and magnesium ions has been studied. Complete directions for an improved method of preparing 8-hydroxyquinaldine are given.

MANY derivatives of 8-hydroxyquinoline, "oxine", have been prepared and their analytical uses have been investigated (7). Most of these derivatives have been 5-, 7- or 5,7 substitution products. Although several 2-substituted derivatives are known, apparently only one, 2-phenyl-8-hydroxyquinoline-carboxylic acid, has been tested for analytical purposes (1). Since the 2-methyl-8-hydroxyquinoline or 8-hydroxyquinaldine has been known for some time (2) and is one of the simplest 2-substituted derivatives, the authors choose to investigate it first. As compared to 8-hydroxyquinoline, 8-hydroxyquinaldine exhibits some important differences in behavior. Probably because of its increased size, it is a more selective reagent. If size of the molecule is a determining factor, the larger molecule might be expected not to react with the smaller ions because of the difficulty in grouping three large molecules around the small ion. If the complex is formed, it might be less stable. This is supported by the fact that 8-hydroxyquinaldine does not react with aluminum ions, one of the smallest trivalent ions (3) with which 8-hydroxyquinoline reacts, and the 8-hydroxyquinaldine complex with ferric ion is precipitated completely only in a much more acid solution than that required by 8-hydroxyquinoline.

## REAGENTS

**2-METHYL-8-HYDROXYQUINOLINE.** The original method of Gebner and v. Miller (2) was modified as suggested by Kochenberger (5). Fifty-five grams (0.50 mole) of *o*-aminophenol and 100 grams (0.18 mole) of *o*-nitrophenol were dissolved in 100 grams of 12 *N* hydrochloric acid in a three-necked flask fitted with reflux condenser, mechanical stirrer, and dropping funnel. Forty grams (0.57 mole) of crotonaldehyde were added with stirring for a period of about 45 minutes. The mixture was heated on a steam bath for 6 hours with continual stirring and was then allowed to stand overnight. The excess *o*-nitrophenol was removed by steam-distillation from the acid solution. Fourteen grams of *o*-nitrophenol were recovered.

The residue was nearly neutralized with 6 *N* sodium hydroxide solution and then saturated with sodium carbonate and steam-distilled. The yield of crude 8-hydroxyquinaldine was 24 to 32 grams or 30 to 40%.

Five grams of the crude material were distilled under reduced pressure (water pump) in a sublimation tube and 4.9 grams of red material were obtained. The 8-hydroxyquinaldine (4.9 grams) was crystallized from a mixture of 20 ml. of 95% ethyl alcohol plus 10 ml. of water and 4.1 grams of a slightly yellow product were obtained, m.p. = 69° C. This product is pure enough for analytical use but was recrystallized once again for the authors' experiments: m.p. = 72° C. (literature, 2, 74° C.) The crude material can be recovered from the mother liquors.

The reagent solution is prepared by dissolving 5 grams of 8-hydroxyquinaldine in 12 grams of glacial acetic acid and diluting to 100 ml. with water. An alcoholic solution is prepared by dissolving 5 grams in 100 ml. of 95% ethyl alcohol. Alcoholic solutions of the reagent turn dark in 1 to 2 days and should be freshly prepared. An acetic acid solution is stable for a week or longer.

**STANDARD SOLUTIONS.** Standard solutions of iron, copper,

and zinc were prepared by dissolving weighed samples of the pure metals in acid and diluting to volume in a volumetric flask. Copper was dissolved in nitric acid and evaporated with sulfuric acid to obtain the sulfate. Iron was dissolved in sulfuric acid and oxidized to the ferric state with nitric acid, the excess of which was later evaporated off. Zinc was dissolved in hydrochloric acid.

A standard solution of magnesium sulfate was prepared by dissolving a weighed amount of c.p. magnesium sulfate heptahydrate in a measured amount of water. The solution was further standardized by gravimetric precipitation of the magnesium as magnesium ammonium phosphate and ignition to the pyrophosphate and by precipitation of the 8-hydroxyquinoline salt.

A standard solution of aluminum ion was prepared by dissolving c.p. potassium alum in a measured amount of water.

**STANDARD POTASSIUM BROMATE SOLUTION.** A standard potassium bromate solution, approximately 0.1 *N*, was prepared by dissolving a weighed amount of c.p. potassium bromate, dried at 110° C., in water and diluting to the mark in a volumetric flask. It was further standardized against Bureau of Standards arsenious oxide, using methyl orange as indicator.

**STANDARD SODIUM THIOSULFATE SOLUTION.** c.p. sodium thiosulfate was dissolved in distilled water to make an approximately 0.05 *N* solution, which was standardized against the potassium bromate solution.

**AMMONIUM ACETATE SOLUTION, 2 *N*.** This was prepared by dissolving 154 grams of c.p. ammonium acetate in water and diluting to 1 liter.

## QUALITATIVE REACTIONS OF 8-HYDROXYQUINALDINE

Qualitative tests were performed upon most of the common ions as listed below. In acetic acid-acetate buffered solutions, 8-hydroxyquinaldine forms precipitates with Bi<sup>+++</sup>, Cd<sup>++</sup>, Cr<sup>+++</sup>, Co<sup>++</sup>, Cu<sup>++</sup>, Fe<sup>++</sup>, Fe<sup>+++</sup>, Mn<sup>++</sup>, Ni<sup>++</sup>, Ag<sup>+</sup>, TiO<sup>++</sup>, Zn<sup>++</sup>, MoO<sub>4</sub><sup>--</sup>, WO<sub>4</sub><sup>--</sup>, and VO<sub>3</sub><sup>-</sup>. It does not form a precipitate with Be<sup>++</sup>, Al<sup>+++</sup>, Ca<sup>++</sup>, Sr<sup>++</sup>, Ba<sup>++</sup>, Pb<sup>++</sup>, Mg<sup>++</sup>, K<sup>+</sup>, Na<sup>+</sup>, or NH<sub>4</sub><sup>+</sup>. It does not precipitate Bi<sup>+++</sup> or Sn<sup>++++</sup> in tartrate solutions.

In ammoniacal solutions, the ions precipitated in acetic acid-acetate solutions, with the exception of MoO<sub>4</sub><sup>--</sup>, WO<sub>4</sub><sup>--</sup>, and small amounts of VO<sub>3</sub><sup>-</sup>, are precipitated and, in addition, Pb<sup>++</sup>, Mg<sup>++</sup>, Ca<sup>++</sup>, and Sr<sup>++</sup>. Aluminum ions are still not precipitated. Tartrate was added to the solution to prevent the precipitation of aluminum hydroxide.

## EFFECT OF pH UPON PRECIPITATION OF ZINC, COPPER, IRON, AND MAGNESIUM

Zinc, cupric, ferric, and magnesium ions were selected for further study as probably the most important and representative ions which are precipitated by 8-hydroxyquinaldine.

A definite amount, 24.99 ml., of the standard solution of one of the four ions, containing, respectively, 0.05121 gram of zinc, 0.05028 gram of copper, 0.04965 gram of iron, or 0.02558 gram of magnesium, was taken for precipitation. An excess of 1 to 2 ml. of 5% 8-hydroxyquinaldine in 2 *N* acetic acid was added and the total volume brought to about 200 ml. with distilled water. The solution was heated to 60° to 80° C. and 2 *N* ammonium acetate solution was added until the desired pH, as determined by means of a glass electrode, was reached. The precipitate was filtered through a Gooch crucible and dried at 120° to 130° C. for at least 3 hours and weighed. The per cent precipitated is plotted against the pH in Figure 1.

According to Figure 1 it appears probable that cupric, ferric, or zinc ions could be separated from magnesium. The separation of each of these ions from aluminum is also a possibility with this reagent. The ferric complex with 8-hydroxyquinaldine requires a considerably higher pH for complete precipitation than the corresponding 8-hydroxyquinoline complex (4, 6).



## RECOMMENDED PROCEDURES

Zinc can be separated from aluminum and magnesium ions by precipitation in acetic acid-acetate solutions with 8-hydroxyquinoline. The zinc can be determined gravimetrically by weighing the precipitate or volumetrically by bromination. If aluminum is present, tartrate is added to prevent precipitation of any basic aluminum salts.

Magnesium can be determined in the filtrate from the zinc determination, if no aluminum is present, by raising the pH to 9.3 or higher. When tartrates and a high concentration of ammonium salts are present (when aluminum is present) the magnesium-8-hydroxyquinoline complex precipitates so slowly that the method is useless. Calcium ions interfere in the magnesium determination in amounts over 2 or 3 mg. and should be previously removed.

Aluminum can be determined after removal of the zinc by adding 8-hydroxyquinoline.

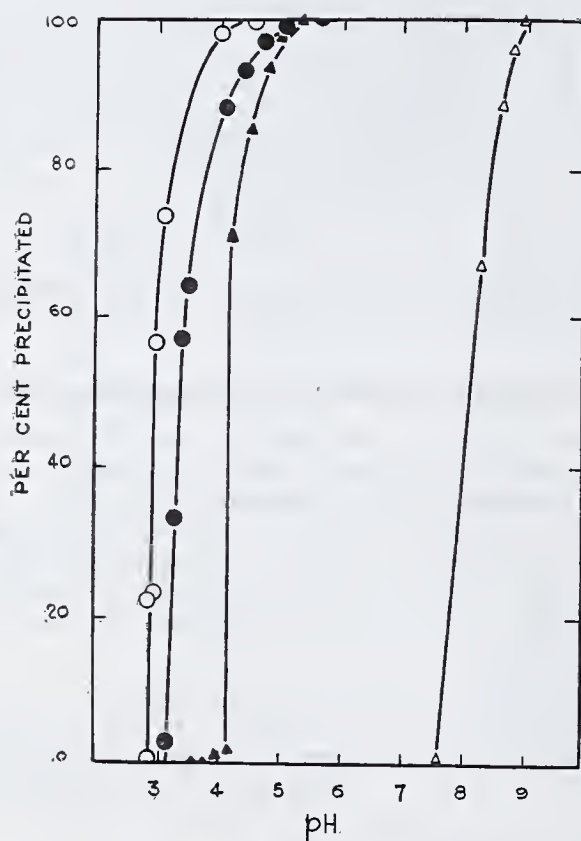


Figure 1. Effect of pH upon Precipitation of 8-Hydroxyquinoline Complexes

○ Cupric  
● Ferric  
△ Magnesium  
▲ Zinc

It is advantageous to use an alcoholic solution of 8-hydroxyquinoline for the volumetric determination of magnesium and an alcoholic solution may be employed in the zinc determination. The reagent is more soluble in the presence of alcohol and is not coprecipitated in the alkaline solutions. No trouble is experienced in the gravimetric determinations and when using acid solutions. The coprecipitated reagent is volatile at 130° C. The magnesium and zinc salts are soluble in hot 95% alcohol and the solubility in water is undoubtedly slightly increased by the presence of alcohol; therefore only the required amount of reagent should be employed. The presence of an excess of reagent is indicated by a yellow filtrate. If the supernatant liquid is not yellow, more reagent should be added.

**PROCEDURE FOR ZINC.** If aluminum is present add 1 gram of ammonium tartrate to the clear, slightly acid solution. Add 2 ml. of 5% 8-hydroxyquinoline solution in 2 *N* acetic acid for every 10 mg. of zinc present, dilute the solution to about 200 ml., and heat to 60° to 80° C. Neutralize the excess acid by adding dilute (1 to 5) ammonium hydroxide drop by drop until the zinc

Table 1. Determination of Zinc, Magnesium, and Aluminum

Zn Taken Gram	Zn Found Gram	Mg Taken Gram	Mg Found Gram	Al Taken Gram	Al Found Gram
Gravimetric results					
0.0512	0.0515	....	....	0.0500	0.0500
0.0512	0.0512	....	....	0.0500	0.0500
0.0510	0.0513	....	....	0.0100	0.0100
0.0510	0.0509	....	....	0.0100	0.0103
0.0510	0.0513	0.0287	0.0285	....	....
0.0510	0.0513	0.0115	0.0112	....	....
0.0205	0.0202	0.0115	....	0.0248	....
0.0109	0.0103	0.0115	....	0.0248	....
0.0020	0.0018	0.0287	....	0.0250	....
Volumetric results					
0.0512	0.0510	0.0256	0.0256	....	....
0.0512	0.0516	0.0287	0.0286	....	....
0.0205	0.0208	0.0256	0.0254	....	....
0.0040	0.0044	0.0287	0.0286	....	....
0.0040	0.0042	0.0287	0.0286	....	....
0.0510	0.0518	0.0287	....	0.0250	....
0.0510	0.0507	0.0287	....	0.0250	....
0.0206	0.0204	0.0287	....	0.0250	....
0.0010	0.0013	0.0287	....	0.0100	....
0.0010	0.0013	0.0287	....	0.0100	....

complex salt which forms on the addition of each drop just dissolves on stirring. Add 45 ml. of 2 *N* ammonium acetate slowly and with stirring. The pH should be at least 5.5. Allow the solution to stand for 10 to 20 minutes before filtering through a Gooch or filtering crucible if the precipitate is to be weighed volumetrically. If the amount of zinc is low and the amount of aluminum and magnesium is high, allow the solution to stand several hours before filtering. Wash well with hot water. If the precipitate is to be weighed, dry it at 130° to 140° C. for at least 2 hours.

To determine the zinc volumetrically, dissolve the washed precipitate with 30 ml. of hot 1 to 2 hydrochloric acid and wash thoroughly with hot 1 to 3 hydrochloric acid and then with water. Moisten the paper with a few drops of concentrated hydrochloric acid before the final two washings with water in order to ensure the complete solution and removal of all zinc complex salt. If the amount of zinc is small and the amount of aluminum and magnesium is large, reprecipitate the zinc as described above. Use only 1 to 2 ml. of the 8-hydroxyquinoline reagent for reprecipitation.

Dissolve the second precipitate in 30 ml. of hot 1 to 3 hydrochloric acid, wash the paper thoroughly with hydrochloric acid and hot water as before, and add 3 grams of potassium bromide to the filtrate. Dilute the solution to about 150 ml. and add a few drops of methyl red indicator. Run in standard 0.1 *N* potassium bromate solution from a buret until there is an excess present shown by the bleaching of the indicator. Add 5 ml. of bromine solution in excess. Add 3 grams of potassium iodide, stir until dissolved, and back-titrate with standard sodium thiosulfate solution using a 2% starch solution as indicator.

If no aluminum is present and no tartrate has been added, the proper pH for the precipitation of zinc can be attained by adding dilute ammonium hydroxide until a white precipitate of zinc hydroxide appears. Redissolve the zinc hydroxide with a drop of acetic acid. Add 2 ml. of the acetic acid solution of 8-hydroxyquinoline for each 10 mg. of zinc present and then 2 to 3 drops of concentrated ammonium hydroxide. The pH should be at least 5.5. This procedure eliminates the high concentration of ammonium salts and makes it easier to reach the required pH for the precipitation of magnesium. If ammonium acetate is used, the least amount possible should be added.

**PROCEDURE FOR MAGNESIUM.** If aluminum was not present and tartrates were not added when zinc was precipitated, the filtrate from the zinc determination can be used for the determination of magnesium. Add 3 ml. of acetic acid solution of 8-hydroxyquinoline for every 10 mg. of magnesium present (if the determination is to be carried out volumetrically, use an alcoholic solution of the reagent) and add concentrated ammonium hydroxide until the pH is at least 9.3 or until no further precipitate forms. Digest the solution at 60° to 80° C. for 20 minutes and filter through a Gooch or filtering crucible if the magnesium is to be determined gravimetrically or through a paper filter if the determination is to be completed volumetrically. Wash with hot water, dry the precipitate at 130° to 140° C., and weigh for gravimetric determination.

The precipitate may be dissolved in hydrochloric acid and titrated with potassium bromate according to the procedure outlined above for the volumetric determination of zinc.

**PROCEDURE FOR ALUMINUM.** After the zinc has been removed, aluminum may be precipitated from the filtrate by adding



droxyquinoline. Warm the filtrate to 60° to 80° C. and add 10 ml. of a 2.5% solution of 8-hydroxyquinoline in 7.5% acetic acid and then 10 ml. of 2 N ammonium acetate. Allow the precipitate to digest 10 to 20 minutes and filter through a Gooch or filtering crucible. Wash with hot water and dry at 130° to 150° C. for at least 2 hours. The precipitate may also be determined volumetrically as described above for zinc.

Results of several typical analyses are given in Table I.

#### ACKNOWLEDGMENT

The authors wish to acknowledge the help of Robert Mosher, who carried out some of the preliminary experiments with this reagent.

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## Determining Phytin Phosphorus

### Stoichiometric Relation of Iron and Phosphorus in Ferric Phytate

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stoichiometric relationship between phosphorus and iron in ferric phytate with an atomic ratio of 6P/4Fe was found to exist when phytic acid was precipitated with a sufficient excess of ferric chloride in the presence of sodium sulfate. On the basis of this finding, a method was developed for the determination of phytin phosphorus in corn grain.

THE determination of phytin phosphorus by the method of Heubner and Stadler (4) is based on the titration of phytic acid with standard ferric chloride solution with the formation of ferric phytate, in the presence of ammonium thiocyanate indicator. The titration is carried out in the presence of 0.6% hydrochloric acid. The end point, shown by the reddish brown ferric thiocyanate, is indefinite and is taken arbitrarily as the point at which the color persists for 5 minutes. It is evident that the nature of ferric phytate and particularly the ratio of iron to phosphorus in it are of vital importance in the evaluation and use of this method.

The gravimetric ratio of phosphorus to iron in Heubner and Stadler's method is 1.19. This factor has been confirmed by Yoe (5). By using Starkenstein's (6) and Anderson's (1, 2) formulas for phytic acid, corresponding to  $C_6H_6O_6[PO(OH)_2]_6$  and  $C_6H_6O_6[PO(OH)_2]_6$ , respectively, it may be calculated that the factor, 1.19, represents the addition of 2.8 moles of iron to 1 mole of phytic acid—that is, the gravimetric ratio 6P/2.8Fe is 1.19. From these formulas it may also be observed that there are 12 hydrogen atoms which theoretically may be replaced by 4 moles of ferric iron. This means, therefore, that the Heubner-Stadler end point does not represent complete saturation of the phytic acid with ferric iron, but that it is merely an intermediate point in the saturation process which is reproducible with a fair degree of accuracy.

The idea of completely saturating phytic acid with iron occurred to the writer as a possible method of determining phytin phosphorus. The data from this investigation, presented in this paper, indicate that 1 mole of phytic acid (inositol hexaphosphoric acid) under proper conditions will add 4 moles of ferric iron.

In this case the relationship of phosphorus to iron in tetra-meric phytate is 6 moles of the former to 4 of the latter. This atomic ratio, 6P/4Fe, corresponds to the gravimetric factor, 1.19. This relation places iron and phosphorus on a chemical equivalent basis in the molecule and obviates the necessity of using an empirical factor. The empirical formula of this com-

pound is believed to be  $C_6H_6O_{24}P_6Fe_4 \cdot 3H_2O$ . However, in view of the polyvalence of both phytate and ferric ions, it is unlikely that any such molecules as above formulated are formed. The probability is very great that the respective ions unite in positions that may be termed "out of phase", with the result that a polymeric type of precipitate is formed with no definite molecular boundaries. The gradual rather than stepwise loss of reactivity with the decrease of replaceable hydrogen on approaching the Heubner and Stadler end point, points to this conclusion as well as the absence of a definite end point. This conclusion is also supported by the nonintegral atomic ratio of 6P/2.8Fe in the precipitate formed at the end point chosen by Heubner and Stadler.

In studying the relationship of phosphorus and iron in ferric phytate, two sources of phytic acid were used—namely, calcium phytate and corn grain extract.

The calcium phytate was obtained from the Soil Biology Department. It was shaken with a large excess of distilled water to dissolve any soluble fractions which might be present. It was then filtered, washed with additional water, then alcohol, and dried at 100° C. for 30 hours. This substance contained 16.09% total phosphorus and from its reaction with iron corresponded to the formula  $C_6H_6O_{24}P_6Ca_6 \cdot 3H_2O + 12H_2O$ . The data in Table I were secured with this material according to the procedure given for the corn grain extract.

The remainder of the data in the paper were secured on phytic acid freshly extracted from corn grain. The freshly ground corn was extracted with 1.2% hydrochloric acid, containing 10% by weight of sodium sulfate, for 2 hours on the shaking machine. The ratio of grain to solvent was 1 gram to 20 ml. The acid ex-

Table I. Phosphorus-Iron Ratios in Ferric Phytate Precipitate

(As influenced by iron-phosphorus ratio in the precipitating mixture. Precipitation from acidified calcium phytate)

In Precipitating Mixture			In Precipitate		
P, Mg.	Fe, Mg.	Multiple of theoretical ratio, 4Fe/6P	Fe, Mg.	P/Fe	Atomic ratio
4.18	8.05	1.6	4.18	1.19 <sup>a</sup>	6P:2.80Fe <sup>a</sup>
4.18	10.04	2.0	4.54	1.000	6P:3.33Fe
4.18	10.04	2.0	4.33	0.921	6P:3.62Fe
4.18	12.05	2.4	4.33	0.965	6P:3.45Fe
4.18	12.05	2.4	4.78	0.874	6P:3.81Fe
4.18	12.05	2.4	4.67	0.895	6P:3.72Fe
4.18	17.31	3.4	4.99	0.838	6P:3.98Fe
4.18	17.31	3.4	4.77	0.876	6P:3.80Fe
4.18	43.27	8.6	5.02	0.833	6P:4.00Fe

<sup>a</sup> Values which correspond with Heubner-Stadler end point; not experimental.



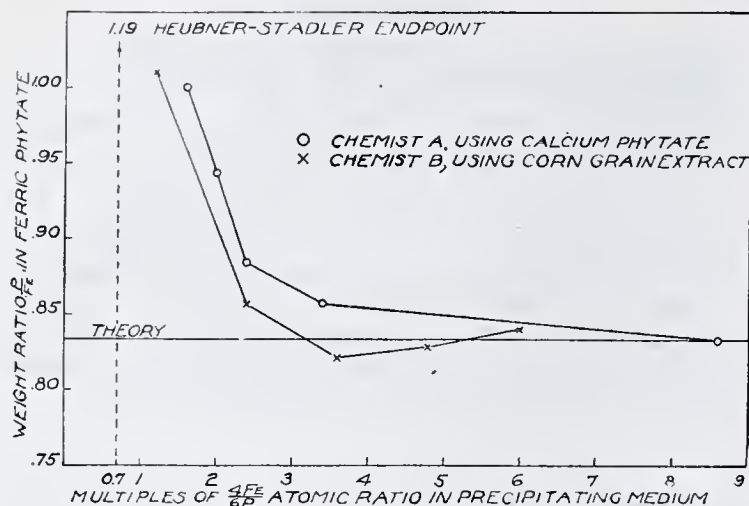


Figure 1. Relation of Concentration of Iron in Precipitating Medium to Composition of Ferric Phytate Precipitate

tract was centrifuged and filtered through a filter paper and asbestos using suction. About 800 ml. of corn extract were prepared. Phytic acid from several aliquots of this stock solution was precipitated with excess iron. The precipitate was filtered on an asbestos mat in a Gooch crucible with suction and washed 5 times with 0.3% hydrochloric acid-2.5% sodium sulfate solution. The precipitate was moistened with a few drops of 50% magnesium nitrate solution and ignited in a muffle for 1 hour at about 1000° C. The asbestos mat and residue were transferred into a 250-ml. beaker and the residue was dissolved in about 10 to 15 ml. of 1 to 1 hydrochloric acid. The solution was filtered and made to 200 ml. with distilled water, and total phosphorus was determined (3). The total milligrams of phosphorus found in the iron precipitate represented the phytin phosphorus (7) contained in the aliquot taken from the stock solution.

The relationship of phosphorus and iron in ferric phytate was obtained by precipitating the phytic acid in aliquots of these stock solutions with a known excess of standard ferric chloride solution for about 1 hour. The ferric chloride solution contained about 0.2% iron and 0.6% hydrochloric acid (4). The total volume also contained 0.6% hydrochloric acid (4) and about 4% sodium sulfate. Excess iron in the supernatant liquid was determined as described below, and from these data the ratio of phosphorus to iron in ferric phytate was calculated.

#### EFFECT OF IRON CONCENTRATION IN PRECIPITATING MIXTURE ON PHOSPHORUS-IRON RATIO IN FERRIC PHYTATE

The quantity of iron which exists in combination with a given quantity of phytic acid should remain constant over a reasonable range of iron concentration after complete saturation has taken place, if a stoichiometric relation exists between phosphorus and iron in ferric phytate with an atomic ratio of 6P/4Fe. Prior to complete saturation of the phytic acid the quantity of iron reacting with phytic acid should increase with increasing concentration of iron in the precipitating solution.

Experimental data concerning this reaction are given in Tables I and II and Figure 1. Table I represents one of the earlier experiments with calcium phytate in which the duplicates do not agree so closely as in the later work with corn extract. These data indicate that in the precipitation of ferric phytate the extent to which the 12 hydrogen atoms in the phytic acid are replaced by iron is determined by the ratio of iron to phosphorus in the precipitating mixture in the range below about 3.6 times the theoretical ratio in the ferric phytate precipitate—i.e., 4 atoms of iron to 6 of phosphorus. At about this ratio ( $3.6 \times 4\text{Fe}/6\text{P}$ ) the hydrogens of the phytic acid are all replaced by iron, with the result that the presence of any larger ratio of iron to phosphorus has no effect upon the composition of the precipitate although it may accelerate its formation.

Nearly all of the first 3 moles of iron are added to the phytic acid with ease. This is indicated by the fact that in the Heubner and Stadler titration the phytic acid removes all the iron from the solution almost instantly until their end point, corresponding to a 2.8-mole addition, is almost reached. The addition of fourth mole of iron to the phytic acid proceeds with increasing difficulty, as is evidenced by the necessity of more than treble the theoretical atomic Fe/P ratio in the precipitating medium in addition to allowing considerable time for the reaction to completion.

Complete iron saturation of the phytic acid, with an atomic ratio of 6P/4Fe, corresponds to a gravimetric ratio, P/Fe-0.833. This is essentially the ratio obtained in the precipitate when sufficient iron was used and adequate time allowed for reaction.

#### COMPOSITION OF FERRIC PHYTATE PRECIPITATE

In addition to the values obtained by analysis of the residue of iron in the supernatant solution, an experiment was conducted in which both the supernatant solution and the ferric phytate precipitate were analyzed for iron and phosphorus. As will be seen in the last two columns of Table III, the mean composition of the ferric phytate precipitate as calculated from the analysis of the supernatant solution gives a gravimetric P/Fe ratio of 0.836, which is in close agreement with the theoretical value of 0.833.

On the other hand, direct analyses of the ferric phytate precipitate gave values for iron somewhat higher than the theoretical, resulting in ratios similar to those reported by Wrensch and Dyer (7), whose paper appeared after this research was completed. It is believed that the high iron values are a result of the difficulty of washing the ferric phytate precipitate free of inorganic iron and that therefore the analysis of the supernatant solution is the technique which should be followed.

#### QUANTITATIVE METHOD FOR PHYTIN PHOSPHORUS

On the basis of the results of this investigation, a method for determining phytin phosphorus in corn grain has been developed.

Table II. Phosphorus-Iron Ratios in Ferric Phytate Precipitate

(As influenced by iron-phosphorus ratio in precipitating mixture. Precipitation from corn grain extract)

In Precipitating Mixture			In Precipitate		
P, Mg.	Fe, Mg.	Multiple of theoretical ratio, 4Fe/6P	Fe, Mg.	P/Fe	Atomic ratio
3.31	4.75	1.2	3.28	1.009	6P:3.30Fe
3.31	4.75	1.2	3.23	1.025	6P:3.25Fe
3.31	9.50	2.4	3.89	0.851	6P:3.92Fe
3.31	9.50	2.4	3.84	0.862	6P:3.87Fe
3.31	14.25	3.6	4.04	0.819	6P:4.07Fe
3.31	14.25	3.6	4.02	0.823	6P:4.05Fe
3.31	19.00	4.8	4.00	0.828	6P:4.02Fe
3.31	19.00	4.8	4.00	0.828	6P:4.02Fe
3.31	23.75	6.0	3.94	0.840	6P:3.97Fe
3.31	23.75	6.0	3.94	0.840	6P:3.97Fe

Table III. Relation of Phosphorus and Iron in Ferric Phytate Precipitate Determined by Two Procedures

Aliquot	P in Precipitate (Colorimetrically) Mg.	Multiple of Theoretical Ratio, 4Fe/6P <sup>a</sup>	Fe in Precipitate		P/Fe in Precipitate Mg. <sup>b</sup>	Atomic ratio
			Mg. <sup>b</sup>	Mg. <sup>c</sup>		
A	3.30	2.4	3.89	4.65	0.848	0.7
	3.30	2.4	3.84	4.70	0.859	0.7
B	3.30	3.6	4.04	4.20	0.817	0.7
	3.30	3.6	4.02	4.20	0.821	0.7
C	3.33	4.8	4.00	4.40	0.833	0.7
	3.33	4.8	4.00	4.35	0.833	0.7
D	3.31	6.0	3.94	4.30	0.840	0.7
	3.31	6.0	3.94	4.60	0.840	0.7
					Mean	0.836
					Theory	0.833

<sup>a</sup> In precipitating mixture.

<sup>b</sup> By analysis of supernatant solution.

<sup>c</sup> By analysis of ferric phytate precipitate.



Table IV. Comparison of Phytin Phosphorus Values

Corn Sample	Weight of Sample Grams	In Precipitate			In Corn Grain		
		Fe <sup>a</sup> Mg. <sup>c</sup>	Phytin P (Fe × 0.833) Mg.	Phytin P <sup>b</sup> Mg. <sup>c</sup>	Total P %	Phytin P %	Phytin P of total P %
46	1.2249	4.05	3.37	3.34	0.312	0.275	88.1
47	1.1948	3.50	2.91	2.88	0.281	0.243	86.5
83	1.1956	3.55	2.96	2.96	0.285	0.247	86.7
86	1.2228	3.99	3.32	3.30	0.312	0.272	87.2
87	1.1901	3.82	3.18	3.24	0.299	0.267	89.3
90	1.1791	3.65	3.44	3.10	0.296	0.258	87.2

<sup>a</sup> Determined by analysis of supernatant solution.<sup>b</sup> Determined by analysis of ferric phytate precipitate.<sup>c</sup> Average of duplicate determinations from acid extract of a single sample.

Weigh 4.6000 grams of finely ground corn grain into a 200-ml. Erlenmeyer flask, add exactly 100 ml. of 1.2% hydrochloric acid containing 10% sodium sulfate by weight, and shake on a mechanical shaker for 2 hours. Decant the supernatant liquid to a centrifuge tube and centrifuge for about 10 minutes. Decant the liquid through a dry filter paper into a dry beaker. Immediately pipet 50 ml. of the extract into a clean, dry 200-ml. Erlenmeyer flask, add 50 ml. of distilled water, and mix thoroughly. Add 15 ml. of standardized ferric chloride solution prepared in 0.6% hydrochloric acid and containing approximately 2% iron. Rotate the flask gently while adding the iron solution. Stopper the flask and continue to rotate until the ferric phytate forms. Then let stand about 1 hour with occasional shaking. Decant the solution through a dry filter paper into a clean, dry beaker or centrifuge the solution. Immediately pipet 50 ml. of this solution into a 100-ml. beaker, bring hydrochloric acid concentration up to 1 *N*, and put through a Walden river reductor in two portions. Rinse the beaker with six 25-ml. portions of 1 *N* hydrochloric acid, allowing each portion to drain almost to the top of the silver column before adding the next. Catch the solution and washings in a 500-ml. Erlenmeyer flask, add about 0.25 gram of sodium fluoride and 3 drops of sodium diphenylamine sulfonate indicator, and titrate the ferrous iron immediately with 0.00895 *N* potassium dichromate (1 ml. = 0.5 mg. of iron). The end point is very sharp, changing from slightly yellow to purple.

From the titration value the milligrams of inorganic iron in the 50-ml. aliquot may be ascertained, and the value, subtracted from the total milligrams of iron originally added to the aliquot, gives the milligrams of iron chemically bound as ferric phytate. This latter quantity multiplied by 0.833 gives milligrams of phytin phosphorus in 1.0 gram of grain.

This method was further checked on samples of corn grain by comparing the phytin phosphorus obtained by the proposed iron method with that obtained from the ferric phytate precipitate. The iron in the precipitating mixture ranged from 3.1 to 3.6 times the theoretical amount required to react completely with the phytic acid (Table IV).

The results of this test indicate that phytin phosphorus in corn grain may be determined as accurately by the proposed iron method as by the determination of phytin phosphorus in the ferric phytate precipitate.

## ACKNOWLEDGMENT

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## Determination of Small Amounts of Sulfate in Cellulose Nitrate and Other Cellulose Esters

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THE relationship between sulfate content and stability of cellulose nitrate has long been recognized (4). In the process of stabilization of cellulose nitrate the sulfate content approaches zero and the determination of sulfate becomes increasingly difficult.

A number of methods for estimating sulfate in cellulose esters have been described. Cross, Bevan, and Briggs (2) used aqua regia to decompose the sample and determined sulfate gravimetrically on the digest. Berl and Bemann (1) and Hake and Lewis (4) decomposed the organic material with alkali. Kullgren (3) decomposed the sample with hydrochloric acid, evaporated the solution to dryness, burned the residue in a combustion tube in a current of oxygen, and absorbed the evolved sulfuric acid in sodium hydroxide solution. Dunncliff (3) oxidized the cellulose nitrate with nitric acid and sodium chlorate and determined the sulfate gravimetrically. Malm and Tanghe (6) decomposed cellulose acetate by refluxing with nitric acid, completed the oxidation with potassium nitrate, and determined sulfate gravimetrically after removing nitrate by evaporation with hydrochloric acid. When the sulfate content of cellulose nitrate or other esters is very low these methods require the use of large samples to provide sufficient amounts of the barium sulfate precipitate for convenient manipulation and the decomposition becomes lengthy and tedious.

A method of analysis which has proved convenient and which gives reproducible results involves decomposition of the cellulose

nitrate with nitric acid to which a small amount of perchloric acid is added after the initial stage of digestion. The sulfate in the digest is determined by a modification of the Morgulis and Hemphill (7) method. Barium chromate dissolved in dilute hydrochloric acid reacts with sulfate ions to give a precipitate of barium sulfate and an equivalent amount of chromic acid which can be determined iodometrically after the excess barium chromate is precipitated by making the solution alkaline with ammonia. This procedure determines total sulfur, but it is assumed that practically all the sulfur in cellulose nitrate is in the form of sulfate. This assumption is implicit in almost all methods for determining sulfate in cellulose nitrate and seems reasonable in view of the processes and materials used in its manufacture.

## REAGENTS

**BARIUM CHROMATE REAGENT.** Prepare pure barium chromate by double decomposition, using solutions containing theoretical amounts of barium chloride and potassium dichromate. Wash the barium chromate thoroughly with 1% acetic acid and then with water and dry. Dissolve 2.53 grams of barium chromate in 100 ml. of 2 *N* hydrochloric acid and dilute to 1 liter.

**POTASSIUM IODATE, 0.01 *N*.** Dissolve 0.3567 gram of pure potassium iodate in water and dilute to exactly 1 liter.

**SODIUM THIOSULFATE SOLUTION, 0.002 *N*.** Standardize against the standard potassium iodate solution at the same time the determinations are titrated.



PERCHLORIC ACID, 60%.

NITRIC ACID, concentrated reagent grade.

STARCH INDICATOR. Dissolve 1 gram of soluble starch (8) in 100 ml. of boiling water.

AMMONIUM HYDROXIDE, concentrated reagent grade.

SULFURIC ACID, 10%.

POTASSIUM IODIDE, crystals which give no test for free iodine.

#### METHOD

Weigh accurately into a 50-ml. beaker a sample of cellulose nitrate containing between 0.4 and 1.2 mg. of sulfate. The sample should not exceed about 2 grams. Place a small stirring rod in the beaker, add 20 ml. of concentrated nitric acid, cover with a watch glass, and heat on a steam bath until the cellulose nitrate dissolves. Add 3 ml. of 60% perchloric acid and heat on a hot plate so that the solution boils gently. Digest until copious white fumes are evolved. If the solution is colored, add about 10 ml. of water and again digest until white fumes appear. Continue the digestion until the volume of solution in the beaker is less than 1 ml. Do not allow the solution to approach dryness, since this leads to low values and may introduce a hazard. Transfer to a 15-ml. graduated centrifuge tube. Wash the beaker thoroughly by using a medicine dropper, being careful that the total volume does not exceed 5 or 6 ml. Add 5 ml. of barium chromate reagent and allow precipitate to form for at least 4 hours or preferably overnight. Make alkaline with concentrated ammonium hydroxide (about 3 ml.). Add water to make the total volume exactly 15 ml., mix well, allow to stand for 1 hour, and centrifuge.

Pipet a 5-ml. aliquot of the supernatant liquid into a 50-ml. Erlenmeyer flask. Add approximately 50 mg. of potassium iodide and 8 drops of starch indicator. Acidify with 10% sulfuric acid and titrate with sodium thiosulfate solution to disappearance of starch iodide color.

If more than 0.4 mg. but less than 1.2 mg. of sulfate is present in the sample, the sulfate content may be calculated from the stoichiometric factor. One milliliter of 0.002 *N* sodium thiosulfate is equivalent to 0.064 mg. of sulfate ( $\text{SO}_4$ ) or 0.021 mg. of sulfur. If there is less than 0.4 mg. of sulfate in the sample, repeat the determination, using a larger sample, since titrations in this range vary with the amount of perchloric acid remaining after digestion.

#### DISCUSSION AND EXPERIMENTAL

Certain details in the procedure have been incorporated to reduce danger of explosions. If perchloric acid which is both hot and concentrated is brought into contact with organic material, a definite explosive hazard exists. By first degrading the cellulose nitrate with concentrated nitric acid and then adding a small amount of perchloric acid this danger is eliminated because the perchloric acid is diluted by the nitric acid. As the solution evaporates, the organic material is oxidized, so that before the perchloric acid has become concentrated the organic material is

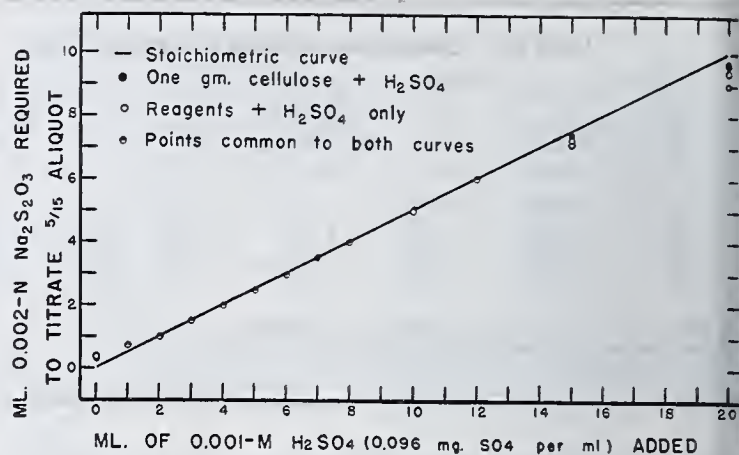


Figure 1. Recovery of Sulfate in the Presence and Absence of Cellulose

decomposed. The solution is never allowed to approach dryness. In the course of several hundred determinations by this method no explosions have occurred.

The range and accuracy of the method were established by weighing a series of 1-gram samples of sulfate-free cotton cellulose into beakers, to which were added definite amounts of a 0.001 *M* (0.096 mg. of  $\text{SO}_4$  per ml.) solution of sulfuric acid. The determinations were then carried out as outlined above. A similar series omitting the purified cellulose was also treated in the same manner. In Figure 1 the values obtained by titration of a 5/15 aliquot with 0.002 *N* sodium thiosulfate are plotted against the milliliters of 0.001 *M* sulfuric acid added to the sample. The stoichiometric curve is drawn on the same figure. When there is less than about 0.4 mg. of sulfate in the sample, the titration values are not reproducible and are usually considerably above theory. In this range the value of the titration is dependent on the amount of perchloric acid remaining after digestion. If 1 ml. or more of perchloric acid is allowed to remain after digestion, titration values considerably greater than those shown in Figure 1 for 0 to 4 ml. of 0.001 *M* sulfuric acid may be obtained. If more than 1.2 mg. of sulfate is present, the titration values tend to be low. Within the range of 0.4 to 1.2 mg. of sulfate the points fall on the stoichiometric curve, so that no deduction for a blank is required for the reagents which were used. This fact should be established for each set of the reagents prepared.

In order to check the accuracy of the method, samples of cellulose nitrate and other related organic materials were analyzed both with and without the addition of known amounts of sulfuric acid. The results, shown in Table I, indicate that sulfate in cellulose esters may be accurately determined by the method. Any ion such as phosphate which forms an insoluble barium salt would interfere. None of these was present in the materials analyzed.

#### ACKNOWLEDGMENT

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Table I. Sulfate Recovery Data

Sample (1 Gram)	Sulfate		
	Added <sup>a</sup> Mg.	Found Mg.	Calculated Mg.
Cellulose nitrate	0.00	1.27	..
		1.25	
	0.19	1.47	
		1.45	1.45
Cellulose nitrate	0.00	0.48	..
	0.48	0.95	
		0.95	0.96
Cellulose acetate	0.00	0.31	
		0.31	..
	0.48	0.77	
		0.78	0.79
Glucose pentaacetate	0.00	0.00	..
		0.00	
	0.48	0.49	
		0.50	0.48
Dextrose	0.00	0.00	..
		0.00	
	0.48	0.49	
		0.50	0.48
Cellulose acetate propionate	0.00	0.27	..
		0.27	
	0.48	0.74	
		0.75	0.75
Cellulose acetate butyrate	0.00	0.09	
		0.10	..
	0.48	0.58	
		0.59	0.58

<sup>a</sup> Added as 0.001 *M*  $\text{H}_2\text{SO}_4$ .



# Photoelectric Automatic Liquid Level Control

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An automatic level control consisting of a photoelectric relay operating a solenoid valve is described. The beam of light actuating the relay is directed at an angle onto the photocell so that the water level, on rising, refracts the beam away from the photocell and closes the solenoid valve.

**ANNUALLY** maintaining a constant liquid level in a forced-circulation vacuum evaporator that had been set up in the laboratory was found difficult. A number of methods of providing automatic level control were considered.

The choice of the type of control to be used is influenced by the following conditions:

The evaporator is operated under a considerable vacuum and the control-actuating device, therefore, must not introduce air leaks to the system.

The level to be controlled is considerably removed from the point at which feedwater is introduced into the system.

The surface of the water is in constant agitation because of the forced circulation and boiling.

It was decided that an on-off control would be desirable because of its simplicity and positive action, its inherent cycling character being no disadvantage since moderate fluctuations in level have no effect on operation of the evaporator.

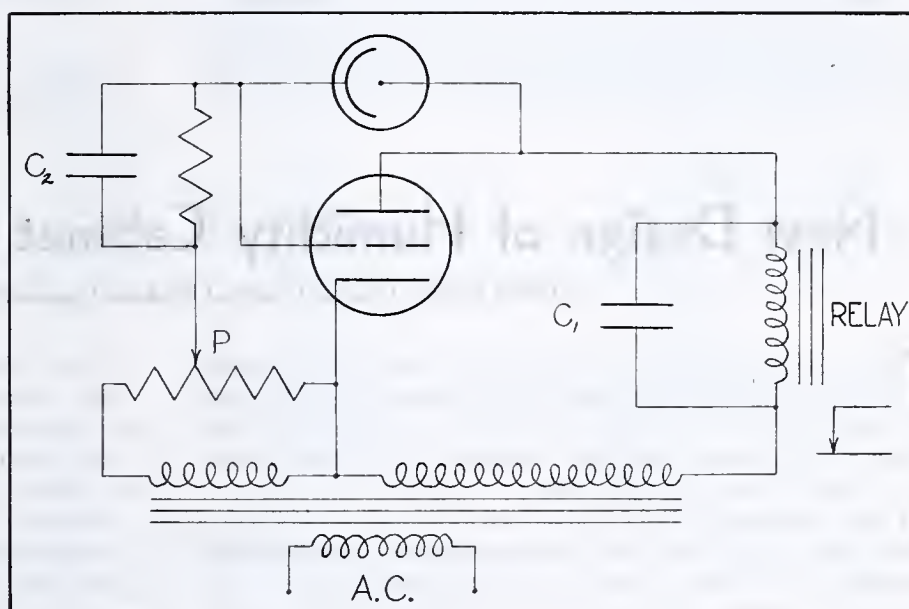
The control which was installed is adapted to operation under the above conditions and consists basically of a photoelectric relay which is controlled by the rise and fall of the level of the water in the evaporator. The relay in turn operates a solenoid valve which controls the feedwater inlet. Sight glasses had already been built into the evaporator and no further vacuum-tight fittings had to be installed.

The water level was made to control the light beam despite its transparency by having the light beam at an angle with the horizontal, so that the beam was refracted away from the photocell by the rising water. A time delay was incorporated into the action of the relay, so that rapid changes in the light beam resulting from continuous agitation of the surface would not affect the relay.

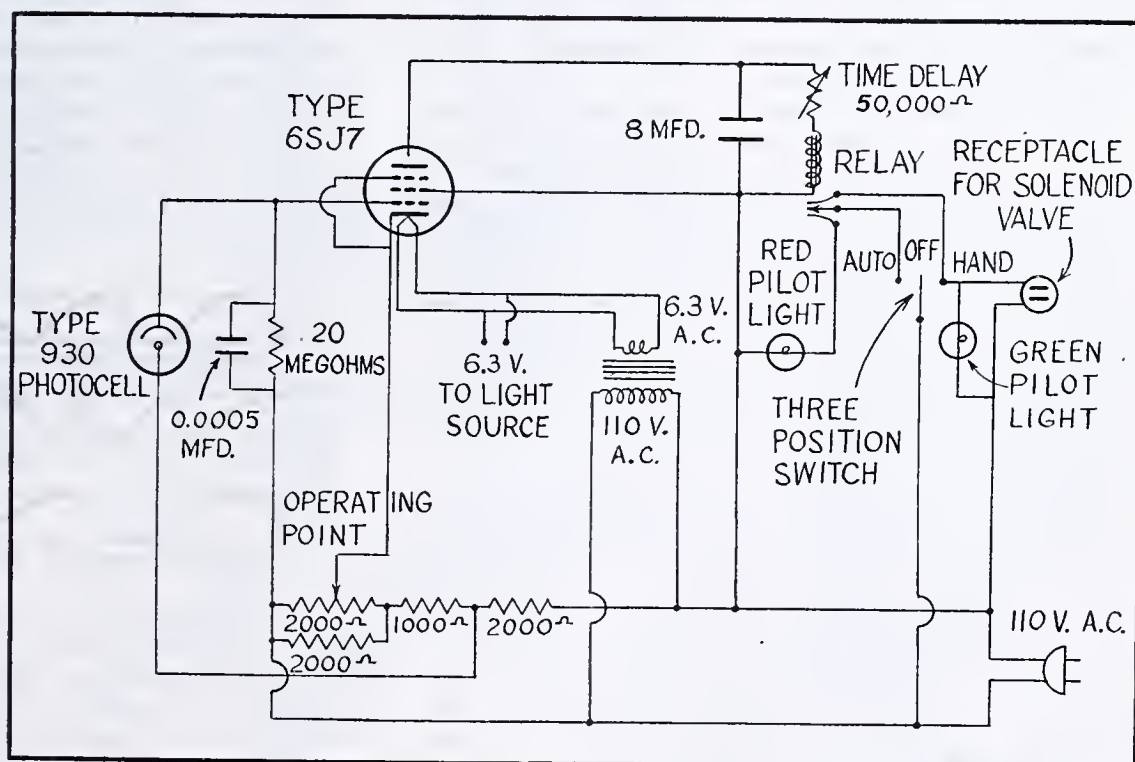
The fundamental circuit of an alternating current-operated phototube relay is shown in Figure 1 (1).

The circuit is that of an ordinary amplifier with the relay as its load. The tube acts as its own rectifier, condenser  $C_1$  preventing the relay from chattering at 60 cycles. Potentiometer  $P$  supplies grid bias for the tube and, with the photocell dark, is adjusted so that the relay has just insufficient strength to close the contacts. When the phototube is illuminated it passes more current and makes the grid less negative, thus increasing the plate current and operating the relay. When the illumination is removed, the relay again opens. Condenser  $C_2$  serves to eliminate the phase difference between grid and plate voltages.

The complete circuit diagram of the photoelectric relay as installed is shown in Figure 2.



### Figure 1. Alternating Current–Operated Phototube Circuit



**Figure 2. Complete Circuit Diagram**

<sup>1</sup> Present address, Midwest Consultants, St. Louis, Mo.



The special transformer appearing in the circuit of Figure 1 is replaced by a voltage-divider arrangement supplying grid bias and the reduced voltage necessary to prevent the gas-filled phototube from glowing. The Type 6SJ7 amplifier tube has suitable plate current to operate the type of relay which was obtained. Time delay is introduced by the inductive-resistive effect of a variable resistor in series with the relay. A three-position switch provides for automatic or hand operation and the two pilot lights indicate whether the relay is open or closed (red indicates valve closed and green valve open). The relay and photocell are wired into the circuit in such a way that the valve will be closed during the period in which the tube is warming up, and also under such extraordinary circumstances as failure of the light source, sudden ebullition because of increased vacuum, etc.

The light source consists of a 32-candlepower automobile headlight bulb fitted with a lens to focus an image of the filament onto the photocell. The solenoid valve was manufactured by the Minneapolis-Honeywell Regulator Company and requires 0.25-ampere steady current at 110 volts alternating current. If the feed enters at atmospheric pressure, the valve should be designed for a pressure of not more than 15 pounds per square inch. If its rated pressure is too high, the valve will not close properly.

**METHOD OF OPERATION.** Place the three-position switch in the automatic position and the "time delay" control at maximum.

Cover the photocell opening with the hand and (a) move the "operating point" control to the right until the green pilot light turns on; (b) slowly move the same control in the opposite direction until the red pilot light just turns on.

The adjustment is critical and should be repeated if the relay chatters or fails to open and close.

This unit has been in operation for over a year and has proved very satisfactory. Anyone with a little radio experience can easily build a control of this type at a total cost of from \$35.00 to \$50.00.

#### PARTS FOR PHOTOCELL RELAY

- 1 SPDT sensitive relay, 1 to 2 ma. to close, contacts rated at 5 amperes noninductive A.C.
- 1 Type 6SJ7 receiving tube (Type 6SJ7GT may be substituted)
- 1 Type 930 gas-filled phototube
- 1 50,000-ohm wire-wound linear potentiometer
- 1 2000-ohm wire-wound linear potentiometer
- 2 2000-ohm, 10-watt wire-wound resistors
- 1 1000-ohm, 10-watt wire-wound resistor
- 1 20-megohm, 0.5-watt carbon resistor
- 1 0.0005-mfd. mica condenser
- 1 8-mfd. 450-volt electrolytic condenser
- 1 transformer, 110 to 6.3 volts at 6 amperes
- 2 110-volt, 6-cp. pilot lights, candelabra base
- 1 6- to 8-volt, 32-cp. Mazda bulb, No. 1132
- 1 SPDT toggle switch, center off position
- 1 2-pole round Bakelite receptacle

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## New Design of Humidity Cabinet for Corrosion Testing

FLOYD TODD, Quaker Chemical Products Corporation, Conshohocken, Pa.

**T**HE corrosion-testing cabinet described herein was designed to give an accurate and reproducible comparison of the relative efficiencies of corrosion preventives which are to be used under indoor storage conditions, including intermediate protection in the process of manufacture.

These conditions differ in kind as well as in degree. For indoor protection the rust-preventive coatings should possess sufficient solubility in suitable solvents to be very easily removed. For outdoor protection it may suffice that the compound be roughly removable, for example, by wiping. Furthermore, for outdoor protection any appreciable water-solubility of the protective agent is very objectionable, as rain would remove any water-soluble ingredients, while for indoor protection it may even be desirable, so long as it does not make the product hygroscopic or susceptible to dew or condensation.

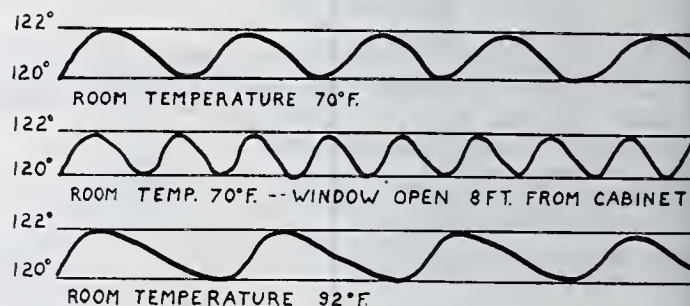
Testing an outdoor type of compound under indoor conditions, or an indoor compound under outdoor conditions, may lead to adopting materials inadequate for the purpose intended, or to discarding products which would have been most advantageous.

This article presents a new testing device for accelerated corrosion tests under extreme indoor conditions (high temperature, high humidity, air exchange, condensation of moisture without washing-out effect, radiation, and such added chemical corrosive influences as may be desired). This cabinet is based on experience accumulated over more than a decade with all types of corrosion-testing devices, and provides a more accurate control of the variables than any other type known to the author.

Cabinets previously used include the conventional type, which consists of a closed space, provided with a spray or bubbling type of humidifier, means for circulating the air, and electrically actuated heater and thermoregulator (4-8). Other types provide for sealed cabinets, operating at high humidity in alternating cycles of higher and lower temperature (Ball Bearing Engineers' Committee). The "weatherometer" is a well-known apparatus adopted for corrosion tests under outdoor conditions (1). Salt-spray cabinets are used for accelerated testing of the relative corrosion proofing efficiencies of protective coatings for metals which may be subjected to sea air exposure (2, 3).

In all prior apparatus the heating has been centralized in too small an area. As a result, convection currents and radiative effects have occurred within the cabinets, causing uneven exposure conditions and consequent serious irregularities in corrosion test results. Furthermore, many humidity cabinets are square or rectangular in shape. Such a design is conducive to undesirable channeling of air and moisture in those cabinets which operate under the usual dynamic exposure conditions.

In any thermostatically controlled apparatus, whether humidity or salt-spray cabinet, the temperature fluctuates within a range of 1° to 3° F. (0.5556° to 1.6668° C.), because of the leakage in the regulator. These variations may be accentuated by convection currents. Even when the temperature fluctuates within constant limits, the actual corrosion temperature ratio may vary greatly, depending on the quality of insulation and the temperature and ventilation of the air surrounding the apparatus. This is illustrated by the following curves, which show the temperature charts in the same cabinet, in which the regulator shuts off the heat at 122° F. and starts heating 120°, a better than average range:



On the upward slope on the temperature curve the air in the cabinet is less than saturated with moisture; on the downward slope it is supersaturated, and condensation may occur. This condensation is irregular and emphasizes any slight surface irregularity of the samples. Moreover, the type of cycle profoundly affects the corrosion behavior, leading to appreciable discrepancies in corrosion time and particularly in corrosion



pes, dependent on external factors not controlled in any cabinet specifications with which the author is familiar. A humidity cabinet for corrosion testing has therefore been designed in order to eliminate the disadvantages of prior cabinets. This design gives additional advantages inherent in only this type of cabinet.

The walls and bottom are heated over a preponderant area. This heating is effected by means of the vapors of a constant-boiling liquid and is therefore absolutely uniform and lagless. Convection currents are thus completely eliminated.

Still more important, the temperature "curve" is here a straight line, the cycles of recurrent sub- and supersaturations are eliminated completely, because the temperature is that of the boiling

point of a constant-boiling liquid, and no mechanical regulation is necessary.

The uniformity of the temperature and humidity conditions within the cabinet give accurate comparisons of the relative efficiencies of corrosion preventives, platings, varnishes, or other protective coatings. These results can be accurately reproduced.

By using cyclopentane as the heating liquid a temperature of 120° F. (49° C.) is maintained, which is a commonly specified condition. By selecting the heating liquid, practically any temperature desired can be maintained exactly.

The air used in the cabinet is preheated to the temperature of the cabinet and prehumidified to 100% before it enters the cabinet chamber, regardless of the operating temperature of the cabinet or of outside conditions.

Air and water entering the cabinet are automatically and accurately controlled and are not affected even by relatively wide variations in the laboratory or plant lines.

The design avoids corners or sharp angles, which tend to cause irregularities in air currents within the cabinet.

The only variable, that of the effect on boiling point of variations of atmospheric pressure, has proved practically insignificant.

#### CONSTRUCTION OF CABINET

The cabinet, shown in vertical section in Figure 1 and in horizontal section in Figure 2, consists of a vapor-tight, jacketed cylindrical container, V, with a conical bottom and top. The chamber is made of No. 22 B. & S. gage Monel metal. The annular spaces and lid, W, are insulated with loosely packed rock wool or glass wool. Fourteen feet (420 cm.) of 0.25-inch (0.6-cm.) copper tubing are bent into a spiral and tacked uniformly around the bottom of the cabinet chamber. The top of the spiral tubing is bent abruptly downward in the central part of the cabinet with its orifice at N. An overflow tube, O, is used to keep a constant

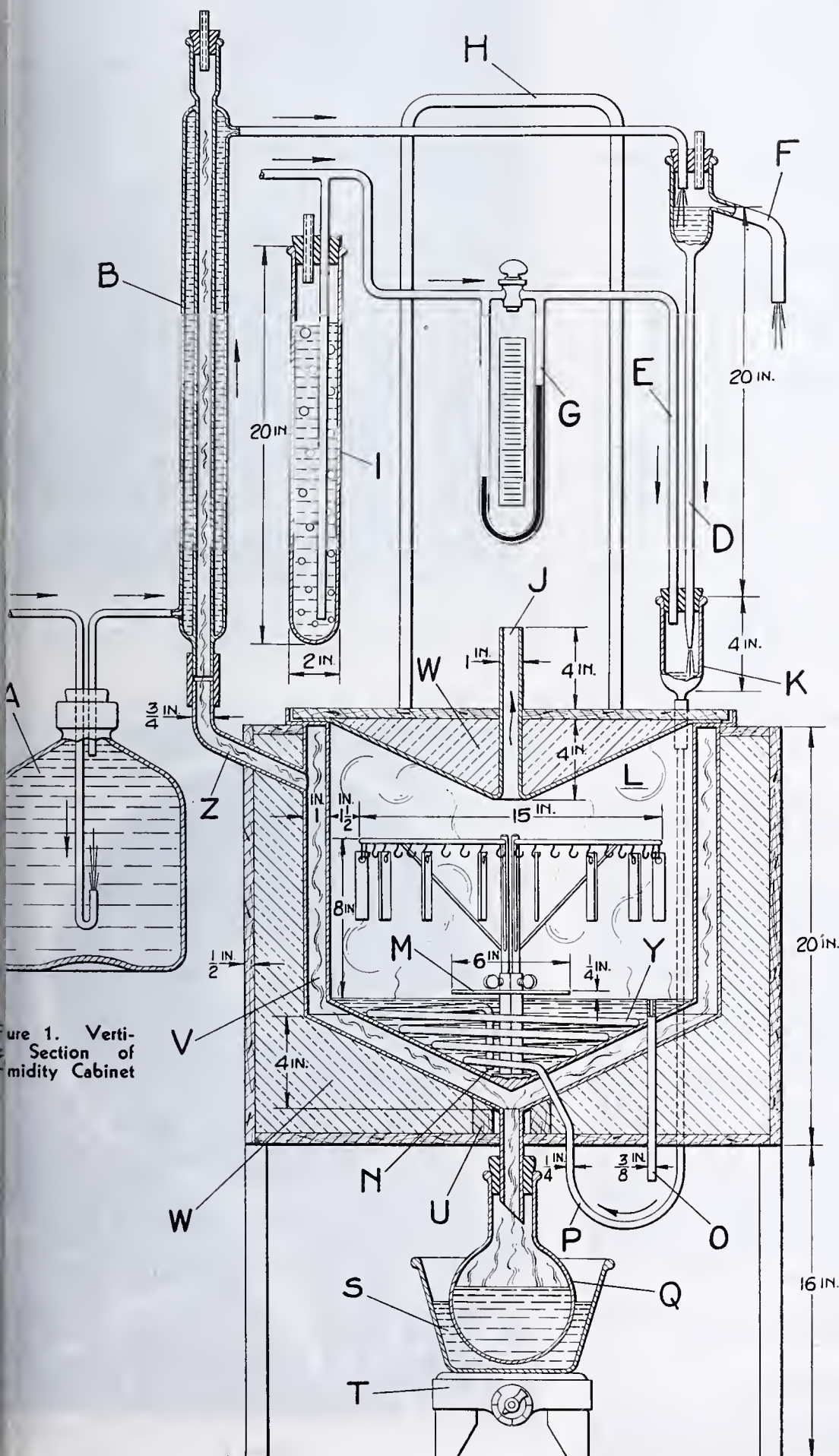


Figure 1. Vertical Section of Humidity Cabinet



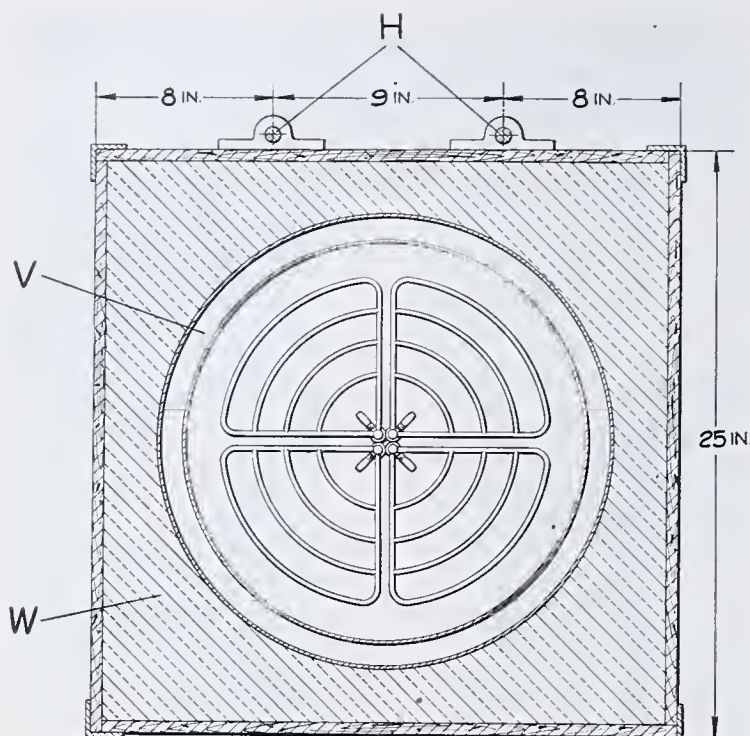
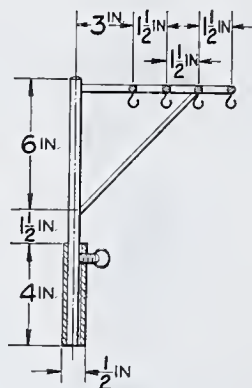


Figure 2. Horizontal Section of Cabinet



water level in the cabinet as shown by the horizontal dotted line. A wooden support, *U*, is used. The cabinet is mounted in a 0.5-inch (1.25-cm.) plywood container. The heating flask, *Q*, is a 2-liter round-bottomed Pyrex flask in an oil bath, *S*.

The test panel supports consist of 4 concentric rings which are divided into quadrants. Each quadrant is a separate unit and may be removed separately for panel inspections. Each quadrant slides into its own vertical support sleeve which is fastened to the center bottom of the cabinet chamber. Panel hooks of No. 16 B. & S. gage Monel metal are spaced 0.625 inch (1.58 cm.) apart on the concentric rings. This allows approximately 50 hooks per quadrant or 200 hooks for the entire cabinet. Experience has shown that the use of these metal hooks instead of glass hooks causes no perceptible corrosion at the point of contact between the panel and the metal hook. The concentric rings to which the panel hooks are supported consist of 0.125-inch (0.3-cm.) Monel metal rod. A baffle plate, *M*, consists of a circular piece of No. 22 B. & S. gage Monel metal and is fastened to the panel support sleeves 0.25 inch above the surface of the water level as determined by the overflow tube, *O*.

Sedimentation bottle *A* has a capacity of 5 gallons (19 liters). Reflux condenser *B* is fairly long with a 0.75-inch diameter inner condenser tube. The top of the condenser is partially closed to decrease loss of the volatile solvent (cyclopentane for b.p. 120° F., acetone for 136°, etc.) by diffusion into the air. The air regulator and safety tube, *I*, is a large 2 × 20 inch glass tube. *G* is an ordinary calibrated flowmeter. *D* is a water-pressure regulator which operates under a constant hydrostatic pressure equivalent to 22 inches. The lower end of this tube is drawn down to a fine capillary, so as to admit a fine stream of water into tube *K*. *H* is an iron support fastened to the back of the humidity cabinet container and is used to hold the control instruments, *B*, *I*, *G*, *E*, and *D*.

Figure 3 is an inside view of the cabinet, taken from above; Figure 4 shows the complete instrument, in operation.

#### OPERATION OF THE CABINET

The cabinet is filled with water to the top of *O*. (Care must be exercised to wash the cabinet thoroughly to remove acid soldering fluxes before using.) This water and the walls of the cabinet

are heated by means of the hot vapors rising from *Q*, at such a rate as to produce slight refluxing in condenser *B*. Because of the good insulation around the cabinet the "low heat" of a small hot plate, *T*, was found to be sufficient.

The humidity in the cabinet is maintained by admitting an excess of water into sedimentation bottle *A*. This bottle allows fine particles of dirt and rust to precipitate, so as not to plug the fine orifice at the bottom of *D*. The water on leaving *A* is used for cooling condenser *B*, then passes into the water pressure regulator, *D*. The excess water is allowed to escape by way of tube *F* to the drain. Water is then automatically admitted at a slow constant rate into mixing tube *K*.

An excess of air is admitted into air-pressure regulator tube. The flowmeter, *G*, is calibrated to allow air, equivalent to 4 times the volume of the humidity cabinet, to pass through tube *E* per hour. The excess air is allowed to escape at the top of tube *E*. This air-pressure regulator tube will keep the rate of air flow into the cabinet constant regardless of relatively wide variations in laboratory or plant air pressures.

The metered air and water in tube *K* descend and enter the bottom of the cabinet at *P*, then travel a long upward spiral path through the submerged copper tubing. During this passage the air is preheated and prehumidified to the same conditions as the humidity cabinet before it emerges at the orifice in the center bottom of the cabinet at *N*. The air then rises vertically through the water in the bottom of the cabinet and finally emerges at the surface beneath the baffle plate, *M*. This baffle plate breaks the air bubbles and in so doing prevents the water spray from the breaking air bubbles from falling onto the test panels. It also serves to distribute the emerging air in a uniform manner throughout the cabinet and thereby prevent air channeling.

The exhaust air is allowed to escape through chimney *J*, which also serves as the handle for removing the lid. The underside of the lid is conical, so as to allow small amounts of water condensing on the lid to return to the bottom of the cabinet by way of the center. In this way the test panels will not be wet by water drops.

When the panel supports are removed for inspecting the test panels, the lid should be replaced as promptly as possible, if a very volatile heating medium is used. Any appreciable drop of temperature inside the cabinet will lead to air entering the jacket and to consequent increase in evaporation losses. These, however, are very moderate. Even with the very low boiling cyclopentane (120° F.) the normal loss is less than 1 pint weekly.

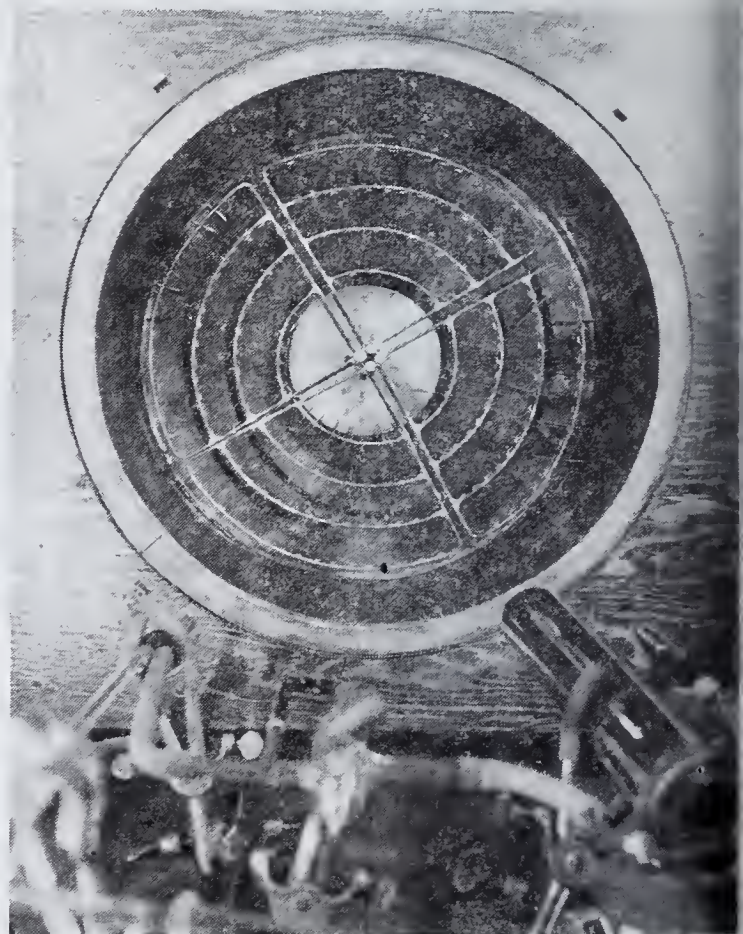


Figure 3



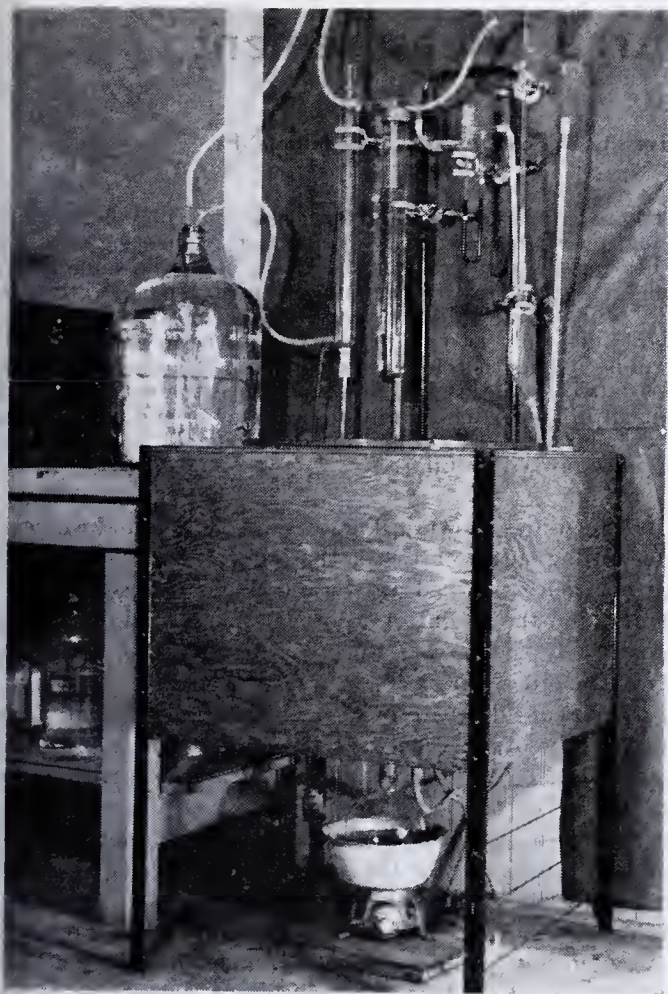


Figure 4

Test panels were suspended in different parts of this cabinet to check its uniformity; regardless of where they were suspended the corrosion was the same. Furthermore, because of the uniformity of the corrosion effects it was found that 1 × 3 inch test panels could be used with results fully as reliable as those obtainable with the usual 2 × 4 inch test panels. These smaller test panels permit a considerably larger testing capacity. Table I illustrates differences in performance between the present cabinet and a cabinet of the conventional type.

Both cabinets were operated at 120° F. and 100% relative humidity. In the conventional cabinet moisture frequently condensed on the panels, and dripped down from them. In the cabinet here described some condensation took place when the test panels were introduced into the cabinet. This initially condensed water remained as a dew on the panels, since evaporation did not take place at 100% relative humidity, but there was no continual condensation, nor any flow of water over the surfaces of the test panels.

The panels used in the test were prepared as follows: Test panels of 1 × 3 inches of S.A.E. 1025 sheet steel B. & S. gage were cleaned by washing in benzene and wiping dry with a clean cloth. Completely new and uniform surfaces were exposed on the test panels by carefully buffing on a cloth buffing wheel (6 inches in diameter × 2 inches thick) coated with an abrasive, such as No. 300 grit Carborundum composition, similar to "Brushing Nu-Glu", as supplied by the J. J. Siefen Co., Detroit, Mich. Care was taken during this buffing operation to remove all sharp burrs at the edges of the test panels. The test panels were again wiped with a clean cloth to remove any traces of polishing grit and were then used. Care was taken not to touch the prepared test panels with the fingers.

All determinations were made in triplicate, and the figures shown are the average values. The variations of the triplicate determinations were within a range of 10% for the new cabinet and 128% for the conventional cabinet.

The fundamental difference between the cabinets is most clearly apparent from the two last items in the tabulation. If rust preventive "Q1" in its experimental stage had been evaluated on the basis of the conventional cabinet alone, it would have been discarded as no more effective than the sodium sulfonate or a fatty acid solution, and only half as effective as the older rust preventive "Q2".

Thus a product highly meritorious in its field of application would have been discarded.

However, the test in the new cabinet showed it to be at least 7 to 10 times more efficient than the petroleum sulfonate or the fatty acid, and at least 3 times more efficient than Q2, under conditions resembling ordinary factory storage, where a frequent flow of water over the surfaces of objects stored is out of question.

Corrosion resistance is a complex phenomenon, and a corrosion preventive which is better under certain test conditions is inferior under other test conditions, and vice versa. The same is true of actual storage under practical conditions.

A corrosion preventive which is most potent in actual use where continuous condensation of humidity takes place may be far from the optimum for storage in even extreme humidities without actual condensation; and a corrosion preventive which is best for the corrosive but not extremely humid atmosphere in steel plants is not best for storage under tropical conditions which are characterized by high temperature and humidity but where corrosive fumes are absent. A detailed analysis of the factors entailed would take us beyond the frame of the present subject; suffice it to say that any accelerated corrosion test must be adapted to the natural conditions in view. The cabinet here described will give an accurate reproducible measure of storage resistance under indoor conditions, in the absence of continuous precipitation or of corrosive vapors. The influence of these latter factors should be measured separately where they play a part, and should not be introduced where they do not enter into the applications envisaged.

Table I. Cabinet Performance

Material Tested	Time Required for First 3 Rust Spots to Appear on Surface of Iron Panels	
	Conventional cabinet Hours	New cabinet Hours
Blank	10 min.	25 min.
Paraffinic mineral oil S.A.E. 40	4	7
10% butyl ricinoleate in Stoddard solvent	24	60
10% methyl oleate in Stoddard solvent	24	80
10% wood rosin in Stoddard solvent	28	65
20% sodium (petroleum) sulfonate in Stoddard solvent	120	236
10% oleic acid in 40 viscosity mineral oil	144	328
20% commercial rust preventive Q1 in Stoddard solvent	120	No corrosion in 2624 hours
20% commercial rust preventive Q2 in Stoddard solvent	240	840

## ACKNOWLEDGMENT

The author takes this opportunity to express his sincere appreciation for the interest and cooperation which Johan Bjorksten, chemical director, Quaker Chemical Products Corporation, has given in the preparation of this paper.

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# Apparatus for Surface Area Measurement

K. A. KRIEGER

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Constructional details and method of use of a compact, rapid apparatus for the measurement of surface areas by low-temperature van der Waals adsorption are described.

SINCE the work of Brunauer, Emmett, and Teller (1) has established the use of van der Waals adsorption as a reliable method for surface area measurements, it is perhaps worth while to describe a simple and convenient apparatus which has been designed especially for such measurements.

## APPARATUS AND PROCEDURE

The apparatus, as shown in Figure 1, consists of four parts: the adsorbent vessel, *A*; the gas buret with four bulbs, *B*<sub>1</sub>, *B*<sub>2</sub>, *B*<sub>3</sub>, *B*<sub>4</sub>; the mercury leveling flask, *D*; and the manometer arm, *E*. Its operation is as follows: With adsorbent in *A* and mercury level at *a*, the adsorbent is degassed through *B*<sub>1</sub>, *B*<sub>2</sub> . . . , and *E*. Then stopcock 1 is closed and the adsorbate—nitrogen, for example—is admitted by the same route. When the mercury level is raised above *a* and *E* is evacuated, the system acts as combined gas buret and manometer and the amount of adsorbate admitted can be calculated from the gas laws. Adsorption occurs when *A* is cooled in a suitable bath—liquid nitrogen, for example—to mark *m*<sub>1</sub> and stopcock 1 opened. By raising or lowering the mercury level to the five engraved marks, *m*, between the bulbs of the gas buret, five points on the adsorption isotherm can be determined.

The volume from stopcock 1 to *A* must be determined by blank runs with *A* both at normal temperature and immersed in the refrigerant.

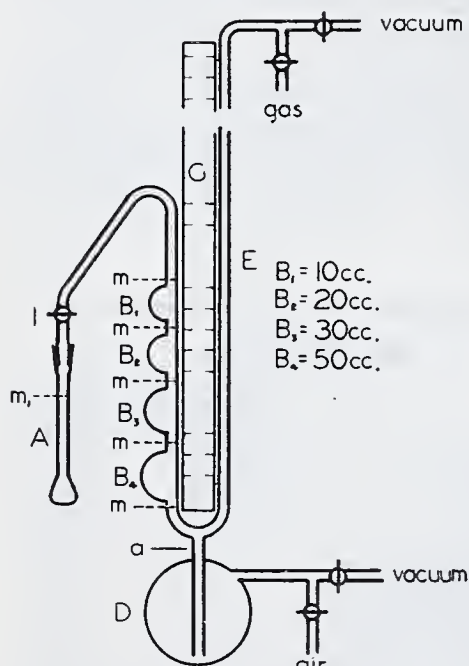


Figure 1. Apparatus

Figure 2 is a plot of the equation of Brunauer, Emmett, and Teller (1) for five bauxite samples prepared by various methods. Areas of the samples can be calculated from  $X_m$  by use of the data given by Emmett and Brunauer (2) and Livingston (5).

## CONSTRUCTIONAL DETAILS

The adsorbent vessel should be conical, so that the adsorbent can be spread in a thin layer on the bottom, since deep layers of active adsorbents must be degassed very cautiously to avoid their being blown out of the vessel by the evolved gases. The use of a ground joint to connect *A* to the measuring system is very convenient, especially if a series of samples of known or constant density is being studied, for then only one "dead-space" determination need be made for the series.

The bulbs of the gas buret are flattened on one side to bring the engraved marks, *m*, between them close to the scale. This is especially important when the apparatus is intended for precision work, since it is then advisable to thermostat the apparatus and read the scale with a telescope. These bulbs should be

constructed of heavy glass to avoid changes of volume due to varying internal pressure. The tubing connecting the bulbs and bearing the engraved marks should be of the same internal diameter as that used for manometer arm *E*—i.e., 7 to 8 mm inside. Since the equation used for calculation of  $X_m$  is a straight line, only two points on the isotherm are required to determine it and therefore only one bulb is actually necessary. At least two are advisable, however, since the extra point thus obtained provides a check on the accuracy of the measurements and calculations. In the author's experience, four bulbs have sometimes proved useful.

The dimensions shown in Figure 1 are those found convenient for measuring surface areas of the order of 100 square meters using nitrogen as the adsorbate and liquid nitrogen as the refrigerant, and the weight of adsorbent taken is adjusted to give approximately that area. Under these conditions a reproducibility of the order of 1 or 2% is to be expected.

If a different adsorbate or refrigerant is used, attention must be paid to the distance between the mark at the top of *B*<sub>1</sub> and the top of scale *C*. This distance must not be less than about three tenths of the saturation pressure for the combination chosen, and if possible should be somewhat more than the saturation pressure. For this reason the bulbs should be set as close together as practicable.

A McLeod or other low-pressure gage should be provided in the vacuum line in order to check completeness of evacuation. Normally it need not be calibrated, since it is used only to prove the attainment of a good vacuum.

## MODIFICATIONS

Obviously this apparatus can be used for measuring ordinary adsorption where the pressures are in the centimeter range and an accuracy in pressure measurement not exceeding about 0.1 mm. is required. It is easily adapted for use with gases soluble

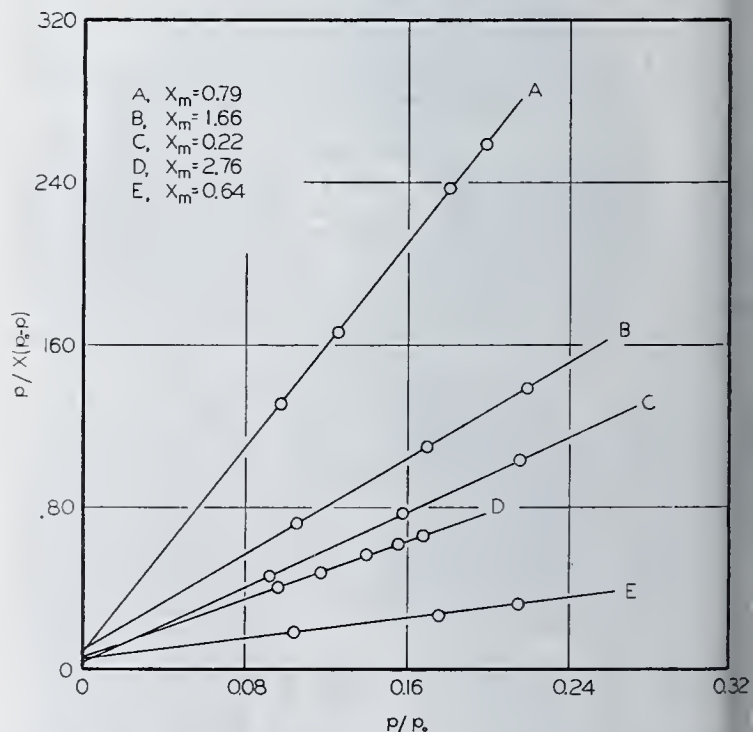


Figure 2. Plot of Equation of Brunauer, Emmett, and Teller for Five Bauxite Samples

$X_m$ , Millimoles of nitrogen per gram of adsorbent  
For curves C and E read 10 times the stated ordinate



is not reactive with stopcock lubricants, since stopcock 1 alone would have to be replaced by another closure (3). The use of a Frittz cut-off, for example, would eliminate both stopcock 1 and the ground-glass joint below it.

#### ACKNOWLEDGMENT

The author wishes to thank the Attapulugus Clay Company for permission to publish the data (4) of Figure 2.

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## Improved Distilling Flask

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THE tendency of many substances to solidify on condensation, thus blocking narrow and inaccessible parts of a distilling apparatus, often leads to interruption of the distillation. To overcome this difficulty Anschütz (1) designed an all-glass distilling unit in which the receiver, acting also as condenser, was welded on to the flask. This construction allowed the removal of obstructing material by heat applied externally. The scimitar-shaped receiver, however, which gives the name of sword flask to this type of distilling flask, was unsuitable for the collection of large amounts of distillate. There was also a risk of contamination by accidental contact with the rubber stopper at the end of the receiver, and complications were encountered when in vacuum distillations the simultaneous use of thermometer and capillary was required.

A few simple changes in the form of the Anschütz flask led to an improved distilling flask (Figure 1) of general applicability which has rendered valuable service over a number of years in distilling substances of low or high boiling point at either normal or reduced pressure. Two of the inconveniences encountered when using the Anschütz flask were overcome by giving the receiver a straight cylindrical shape of greater diameter and an outlet tube. These alterations, without unduly enlarging the receiver, considerably increase its capacity and prevent accidental contamination of the distillate by rubber. To accommodate separately a thermometer and a capillary a short side arm opposite the receiver and at the same height has been added. The thermometer and the capillary tube are held in place by rubber stoppers. If rubber must be avoided, the distilling flask shown in Figure 2 can be used with advantage, as it is especially designed for the distillation of corrosive substances. Both flasks, sturdy by virtue of design, are speedily assembled or dismantled

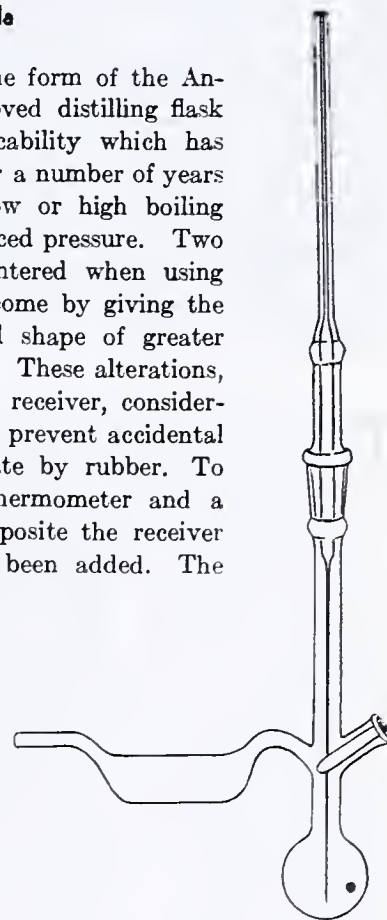


Figure 2

Figure 1

Table I. Dimensions of Flask

Capacity of Receiver	Capacity of Receiver	Neck		Distance of Delivery Tube and Side Arm from Bulb	Receiver		Side Arm		Outside Diameter of Distal End of Receiver
		Outside Diameter	Length		Outside Diameter	Length	Outside Diameter	Length	
Cc.	Cc.	Cm.	Cm.	Cm.	Cm.	Cm.	Cm.	Cm.	Cm.
4	4	1.2	6.5	1.5	1.6	3.0 <sup>a</sup>	1.2	2.2	0.8
10	10	1.6	8.5	2.0	1.8	3.5	1.5	2.5	0.8
30	30	1.6	11.0	3.5	2.5	5.0	1.5	3.0	0.8
60	60	1.6	12.0	4.5	3.2	6.0	1.5	3.5	0.9
100	160	2.2	13.5	5.5	4.4	8.0	1.7	5.0	1.0
50	300	2.5	15.3	5.5	5.0	11.0	2.1	5.0	1.0
						12.0			
						16.0			
						20.0			

<sup>a</sup> Measured along lower edge of receiver.  
<sup>b</sup> Measured along upper edge of receiver.

and easily cleaned. The approximate measurements for the different sizes of the distilling flask (Figure 1) commonly used in this laboratory are listed in Table I.

Ace Glass, Inc., Vineland, N. J., will stock this improved sword flask after the war, and at present is willing to fill any specific order for this item.

#### ACKNOWLEDGMENT

The author wishes to express his thanks to Miss Marjorie Muir, Royal Ontario Museum, Toronto, for preparing the drawings.

The sealed-in capillary and thermometer jacket were added by J. C. Sowden, Toronto.

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# Ultramicrodetermination of Arsenic by Gutzeit Spot-Filtration under Vacuum

## A Rapid Technique Employing Photometric Calibration and Permanent Photographic Standards

HENRY S. SATTERLEE AND GERTRUDE BLODGETT, 242 East 62nd St. and 802 Lexington Ave., New York, N. Y. -

Deficiencies existing in various forms of Gutzeit procedure are examined in relation to the problem of developing a type of end reaction suited to quantitative determination of arsenic in the minute order of 0.04 to 1 microgram. To cover this range, a vacuum-accelerated Gutzeit reduction system for mercuric bromide spot filtration has been designed which prevents sources of error and shortens time of operation. This is supplemented with photoelectrically standardized photographic reference scales, adapted to either visual or photometric evaluation of the spot reactions, an improvement in Gutzeit technique which contributes precision and uniformity to end determinations and abolishes the time and labor required for frequent preparation of fresh standards. Immediate fractional treatment, by distillation and oxygen-bomb combustion of residues, is recommended as the method of choice in preparing fresh biological material for Gutzeit reduction, to prevent possible preliminary losses, and to separate "volatile" from "fixed" arsenic when analyzing such material.

THE reported progress in agricultural and food chemistry during recent years indicates that no method for determination of arsenic in organic material has yet proved satisfactory for routine application to the ultramicro range—for quantities ranging from 0.04 to 1 microgram of the elementary substance.

In the much higher range of 10 to 30 micrograms, the official method as prescribed (1) by the A.O.A.C. is undoubtedly adequate for the main purpose intended—the testing of fruit skins for spray residues—where the optimum range is stated to be from 20 to 25 micrograms of arsenic trioxide. But, even in this bracket, where the inherent error is estimated (6) as between 5 and 10%, stress is laid on the need for strict uniformity of operation throughout the entire procedure in order properly to evaluate the lengths of the paper-strip reactions as finally measured against a standard graph. This precaution denotes the main technical deficiency, which is common to all Gutzeit methods that yield an attenuated pattern of end-reaction as produced by flow of the reagent gas over narrow and elongated surfaces of the sensitized medium.

The task of extending application of the Gutzeit reaction to a more minute working order, while pursuing a "strip" or "string" technique, has embraced many perplexing difficulties, due to uncertainties in obtaining uniform sensitization of the paper, as noted by Cassil (6), and to other variable factors as discussed by Wichmann (25-27), and as instanced in a study of this problem by How (13) and reviewed by Wichmann (26). The prospect has consequently been suggested that less delicate end reactions for arsenic, such as the cerulean-molybdate colorimetric methods of Klein and Vorhes (16) or of Hubbard (14), or even the iodine titrimetric methods (7, 8), might more easily be adapted to ultramicro extension than the Gutzeit method.

Investigation of fresh and unstable organic material in the field of clinical pathology, immunology, and physiological chemistry has demanded the use of an ultramicromethod for quantitative determination of arsenic in the very minute order of 0.04 to 1 microgram. This limitation is due to the small size of test samples of biological material which it is necessary or desirable to examine in this field: blood, glandular secretions,

cerebrospinal fluid, 1 to 2 ml.; urine, liquid culture media, etc., 5 to 10 ml.; expired air, 5 to 10 liters; animal danders, bacterial allergens, etc., 15 to 25 mg.; vegetable pollens, 25 to 50 mg.; house dusts and tobacco, 25 to 200 mg.; fresh biopsy and necropsy tissues, 100 to 500 mg.

Accuracy in analyzing such small samples of organic material for contained arsenic requires:

1. Avoidance of errors due to unsuspected changes of arsenic content during preparatory processes, whether such processes involve the laborious procedures of wet-oxidation or the more rapid procedure of dehydration followed by flame combustion in oxygen.
2. A method of isolation and final determination which will minimize reagent impurities and be capable of showing clearly the presence of 0.01 microgram of elementary arsenic, while regularly giving blank determinations below 0.04 microgram and provided with a system of evaluation having a margin of error not exceeding  $\pm 3\%$ , or visually perceptible differences of values of 0.05 microgram in the optimum range up to 0.50 microgram, and with opportunity for further refinement by photoelectric photometry.

Unless error be avoided under the first category, it will be useless to achieve the precision contemplated under requirements of the second. The present paper, being mainly concerned with a new method and apparatus for attaining objectivity of the second category, does not dwell upon preparatory steps except to express confidence in the merits of performing primary distillation or evaporation in a closed system as against the immediate processing of biological material by open methods of wet-oxidation. The grounds for this preference were reported some years ago in collaboration with Carey (4), and the opinion then expressed has been confirmed by later observations. Findings, accumulated while dealing with the problem of fresh mammalian blood and other thermolabile substances of organic origin, point to the existence, in variable proportions and characteristically minute concentrations, of two forms of arsenic in association with such material: (1) a loosely bound component which is, or becomes, volatile under such natural influences as pulmonary and/or cellular respiration and by exposure to atmospheric evaporation, and which may be artificially separated from such material by drying or distillation at 56° C. in a current of molecular oxygen; and (2) a relatively fixed or thermostable component which is not removed from the material by heating at 56° to 80° C. under the same conditions.

Since these observations derive largely from investigations of clinical material, they will be reported in detail elsewhere and are here briefly summarized only to serve as a premise for pointing out that the ultramicrodetermination of arsenic which is found in association with biological substances may be of physiological interest and not necessarily confined to toxicology.

The authors' interest has been mainly concentrated on arsenic transformations in organic material under natural conditions, as through oxidation-reduction phenomena in physiological systems or through simple exposure to atmospheric oxidation, and not on the artificial effects of destructive analysis by means of powerful oxidizing agents.

The mechanism of dissociation of minute amounts of arsenic by evaporation at 56° to 60° C. and its recovery from fresh biological material by acid extraction of the vapors is not clear since the composition of the volatile product has not yet been



etermined. It is presumed to be derived by the splitting off of a volatile arsenic group from an arsenoprotein, but whether this is an arsenium group, an alkyl arsine, or simply arsenic trihydride is not known. The arsenic content of the volatile fraction in proportion to that of the dried residue of human blood, is very variable, and seems to depend on age and activity of the organism, or more specifically on body metabolism. For example; 212 analyses of human blood taken from 51 adult individuals, the average "total arsenic" found was 59.14 micrograms per 100 cc., ranging from 10 to 190 micrograms, with the proportion of "volatile arsenic", as defined above, averaging 40.57% and varying from 0 to 100%. These variations were, of course, greater for the entire series than for any individual of the group, which was composed of 33 ambulant patients, suspected or definitely diagnosed as "chronic arsenical poisoning" or "arsenic retention", 13 cases "for diagnosis", 2 cases of "hyperallergic state", and only 3 "normal" healthy individuals. Of these latter, 2 had an average "total arsenic" in their blood of 25.5 micrograms per 100 cc. with 85% "volatile", and the third normal individual (in the 28th week of pregnancy) showed a total blood-arsenic of 180 micrograms per 100 cc., which corresponded to the physiological increase during gestation, as shown by Guthmann and Grass (2). In this last case the interesting features were that the "volatile arsenic" fraction was 100%, and the expired-air test, performed at the same time, showed 50 micrograms of arsenic per 100 liters as collected directly through the vacuum extraction train, and was the highest test on exhaled air which has been recorded in the authors' series.

There can be no doubt that the sensitiveness of the Gutzeit arsine-mercuric bromide end reaction is, per se, sufficient to meet the exacting requirements indicated above. There are, however, certain faults in the accepted Gutzeit techniques as affecting ultramicrodeterminations which demand correction in order to extend its usefulness to that range.

**PAPER-STRIP METHOD.** After several years' experience with Gutzeit reactions produced by the paper-strip method it became evident to the authors that the inaccuracies and disadvantages of this system of end reaction, as compared with the paper-disk method, are attributable to: (1) difficulties in appraisement of areas representing less than 2 micrograms, because of unequal distribution on opposite sides of the strip and inconspicuous insensitization along the free edge of the paper and (2) instability of reactions on the "standard" test strips used as criteria for quantitative evaluation. Rapid fading, which may escape attention in the higher ranges, is very noticeable in the low values and renders such strips unreliable as scales of reference within a few hours. Here follows a disadvantage common to both strip and disk systems—the labor and time involved in frequent production of fresh standards for comparison.

**DISK METHODS.** Faults which are particularly attributable to the various Gutzeit disk or diaphragm methods proceed mainly from the heating process at the hydrogen generator, a necessary feature with all positive-pressure systems of disk filtration in order to obtain a prompt and clear-cut reaction upon the sensitized medium. This applies not only to the smaller (5-mm. diameter) areas and to the standard 6.5-mm. disk which is prescribed by the British Pharmacopoeia (3) but to methods (17) employing filtration areas as large as 20 mm. in diameter. Boiling or lesser heating of the zinc-acid mixture is undesirable since not only causes irregular action with fluctuating pressures within the confines of a small generating vessel having a resistant outlet, but produces an uneven flow of gas and a tendency to excessive heat at the absorption level. This has in some instances been lessened by use of a manometer side arm attached to the generator which provides some cushioning effect on pressure oscillations. In other forms of apparatus (17) irregularities are lessened while the total pressure within the system is raised, by forcing hydrogen or nitrogen gas into the generator from outside the system.

Some analysts (11) place a heating limitation of from 40° to 60° C. in the reducing vessel and aim to promote a rapid evolution of hydrogen gas by means of catalytic "impurities" in the acid reagent; either accidental ingredients, or purposely employed with the zinc, as for example, 0.3% of copper (24). Other analysts (10, 20) insist on a relatively slow rate of gas evolution and rely mainly on a stannous chloride activation of the zinc for augmenting activity of the hydrogen gas so produced.

Gutzeit disk systems under varying degrees of positive pressure, commonly practiced, thus appear to present a dilemma, in that they require heat to accelerate gas formation and to maintain sufficient pressure to force a current of gas through the filter medium against the internal resistance of the system plus at-

mospheric pressure beyond the filter disk; while, on the other hand, this same internal pressure factor works against the evolution of gas at the source and raises the boiling point in the generator. Irregular gas production thus becomes inevitable and results in an intermittent or irregular delivery of gas at the reaction level of the filter disk, while excessive heat in the generator carries water vapor to all levels. These conditions tend to wash away some of the water-soluble arsenic-mercury halide stain after its deposition on the filter-disk, causing a marginal diffusion of the reaction or uneven staining. Such effects are lessened, however, by cooling devices placed at or just below the absorption level.

Finally, there exists in all Gutzeit systems which operate under positive pressure the possibility of leaky joints permitting small losses of arsenic trihydride to the atmosphere. Alleged (15) partial losses of arsenic hydride at the absorption level by passage through the sensitized filter disk, with failure to react on the mercuric halide, probably do not occur in ranges of concentration below 10 micrograms, even under boiling conditions, as was shown long ago by Bird (2) and later by Cribb (10).

While most of these objectionable features have been stressed by early workers (23), recognized by later critics (19, 20) of Gutzeit technique, and to some extent corrected as affecting the semimicro range of determination, the authors have found them seriously detrimental in the micro range and a complete obstacle to successful practice in the ultramicro range.

After experimenting for more than a year with various forms of electrolytic cells in seeking to produce atomic hydrogen under instrumental control, the complicating factors so introduced, especially the unpredictable influence of overvoltage upon the more sensitive (mercury) types of electrodes, led the authors to abandon hope of finding a completely reliable electrolytic method and to concentrate upon a vacuum system as the best means for correcting the faults of the zinc-acid generator. They then discovered that a system of spot-filtration securely closed against leakage could be ensured under a low vacuum, and that, when operated at from 0.25 to 0.5 atmospheric pressure, this system would yield more uniform and prompt end reactions with better control of heat and moisture at the absorption level than could otherwise be produced upon a Gutzeit-sensitized medium. By this means, under instrumental control, they furthermore found that the development of active hydrogen in the usual zinc-acid mixture is markedly increased and that the whole reduction process proceeds evenly to a conclusion within 15 minutes. Gas-current impedance in the scrubber, and at the filter disk, is overcome by this device and the end reaction is rendered in sharp demarcation upon a precisely measured circular area of the sensitized paper. For ultramicrodeterminations, the reactive area may be reduced as low as 3.17 mm. (0.125 inch) in diameter, or it may be suited to any preferred range of operation by adjusting the caliber of the filtration jet to 6.35 mm. (0.25 inch) or larger, with corresponding adjustment of the vacuum.

Realization of these characteristics finally provided an opportunity for applying photographic and photometric methods to quantitative evaluation of the Gutzeit end reaction, a project found impracticable with either strip or thread reactions. The use of photographic step scales for permanent standards of reference, as here reported, has now been practiced in this laboratory for more than two years; it is so far beyond the experimental stage that the authors do not hesitate to recommend it as an important time- and labor-saving device which also implements precision in the procedure here described. These artificial standards of reference consist of an accurately graded series of silver deposits photographically printed on bromide paper from a master negative. The fineness of gradation may be varied to suit special requirements (or limited ranges), as is shown in Figure 4.

#### REAGENTS

**SULFURIC ACID**, C.P., special arsenic-free (supplied by the J. T. Baker Chemical Co.), obtained in 500 ml. Pyrex bottles, usually rated on label as having less than "0.000000% As".



Tolerance: 10 cc. in 90 cc. of double-distilled water with 5 cc. of potassium iodide solution, 7 grams of zinc and 0.5 cc. of stannous chloride solution in reduction system under vacuum of 0.33 atmosphere (250 mm. of mercury) for 30 minutes and with intervention of lead acetate solution in absorption tube for removal of hydrogen sulfide, should show less than 0.02 microgram of arsenic, or 0.0000002% of arsenic in a 10-cc. sample of acid.

**HYDROCHLORIC ACID**, c.p. special arsenic-free (J. T. Baker Chemical Co.), obtained in 500-ml Pyrex container as above, and usually rated as not more than "0.0000001% As". Tolerance: 10 cc. in 90 cc. of double-distilled water with 5 cc. of potassium iodide, 7 grams of zinc and 1 cc. of stannous chloride under vacuum in Gutzeit apparatus, as above, should yield a similar blank.

**ZINC METAL**, c.p. special, mossy, for microdetermination of arsenic (J. T. Baker Chemical Co.). The mossy variety of zinc is preferred because it seems to be more consistently free from arsenic than the "granular 20-mesh" variety, although both are claimed by the manufacturers to contain less than "0.000001% As". Tolerance: 7 grams, after preliminary treatment as below for 5 minutes to remove any possible surface contamination from atmospheric adsorption, should be submitted to same tolerance test as hydrochloric acid above and should show less than 0.02 microgram of arsenic. Approximately 7 grams should be used in each arsenic determination having 100 cc. of acid solution in the reduction flask. Such portions, before use for arsenic determination, should always be freshly cleansed, activated with 50 cc. of hydrochloric acid diluted 1 to 3 with double-distilled water and with admixture of 2 cc. of stannous chloride for 5 minutes under vacuum, and then washed in double-distilled hot water. Just before use in a determination the zinc should again be washed in double-distilled water.

**DOUBLE-DISTILLED WATER**. This should give blank test above with the other reagents.

**POTASSIUM IODIDE**, c.p., 15 grams dissolved in 100 cc. double-distilled water. This solution in 5 cc. amounts should give a blank test as above with the other reagents.

**STANNOUS CHLORIDE**, dihydrate, arsenic-free. A 40% solution of this in hydrochloric acid is required. Tolerance: 1 in 10 cc. of hydrochloric acid and other reagents and conditions as stated under hydrochloric acid should yield blank test in the same order.

**LEAD ACETATE SOLUTION**, 10 grams of lead acetate trihydrate in 90 cc. of double-distilled water, made acid to litmus with acetic acid and then made up to 100-cc. volume. One to 3 cc. of this solution poured upon 15 grams of granular Pyrex absorption tube (see apparatus), with reaction conditions described under other reagents, will involve some seepage in the reduction flask. It should yield a blank reaction in the same order.

**PYREX, GRANULAR**, obtained from the manufacturer in fractions between 10- and 20-mesh. This should be sifted to size and then thoroughly cleaned with nitric acid, rinsed with sodium hydroxide solution, and washed with hot distilled water, then dried in an electric oven before use. Under conditions of repeated use it is best left in the apparatus, a 15-gram portion being placed in the hydrogen sulfide-absorption chamber (2, Figure 1) and cleaned after each run (or when blackened by deposition of sulfur) by saturating with 10% nitric acid, neutralizing, and washing.

**PYREX, FIBER**, known commercially as No. 719. A small pledget, previously impregnated with lead acetate solution and allowed to dry, is placed in the absorption tube on top of the Pyrex granular preparation.

**MERCURIC BROMIDE**, c.p. A 5% solution in 95% ethyl alcohol is used for sensitization of the filtration disks as described below.

**WHATMAN NO. 40 ASHLESS FILTER PAPER**, made by Balston & Co., Ltd., black label, cut into disks with a 1.4-cm. ( $9/16$ -in.) punch and sensitized as directed below.

**AMMONIA SOLUTION**, 10%, made by adding 10 cc. of concentrated ammonium hydroxide (d. 0.880) to 20 cc. of 95% ethyl alcohol, in a dropping bottle.

**STANDARD VOLUMETRIC SOLUTION OF ARSENIC**. This is made by dissolving 1.3204 grams of c.p. arsenic trioxide in 1 ml. of a 20% arsenic-free sodium hydroxide solution, saturating this with carbon dioxide, and diluting with double-distilled water to exactly 1000 cc. in a volumetric flask at the standard temperature. From this stock solution, which contains 1 mg. of elementary arsenic (atomic weight 74.91) per cc. at the standard temperature, further dilutions are made with the precaution of sodium sulfite reduction just before use, as recommended by How (13). Dilutions to a final titer of 0.1 microgram of arsenic per cc. are made for use in the ultramicroanalysis of the standard step scales; these dilutions must be conducted with the utmost precision.

**SENSITIZATION OF FILTER DISKS**. A supply of the filter disks, enough for a week or 10-day period, is placed in a wide-mouthed bottle with tubulated stopper, or in a micro filtering flask, and this is half-filled with the mercuric bromide solution. The disks are thoroughly impregnated with the solution and the air is removed from the pores of the paper by exhaustion under vacuum of 75 to 100 mm. of mercury for 2 hours according to the method of Cassil (5). The mercuric bromide solution, unless recently made, should be filtered through a folded filter as practiced by Rosenfels (22) before being used; it should be discarded entirely when 2 months old. The sensitized disks, in an amber-colored bottle, holding the solution, may be safely kept for a week or 10 days if protected from bright light. Just before use they are removed from the bottle, dried in the air on filter paper, and used very promptly; at this stage they should again be protected from bright light and never returned to the bottle, but discarded if not used. Optimal sensitiveness and the proper degree of porosity depend on careful technique in all these details.

For testing the sensitized disks for undue porosity and vacuum-filtration or permeability to excessive amounts (2 to 10 micrograms) of arsenic in the test solution, the authors have superimposed a secondary or telltale disk above the primary disk, but never have observed any sign of arsenic trihydride leakage or telltale staining.

#### APPARATUS

The apparatus is diagrammatically shown in Figure 1. I. A laboratory set up for operation in duplicate units is illustrated in Figure 2.

The hydrogen generator is a 200-ml. Erlenmeyer Pyrex flask with a 24/40 ground joint for connection with the hydrogen su-

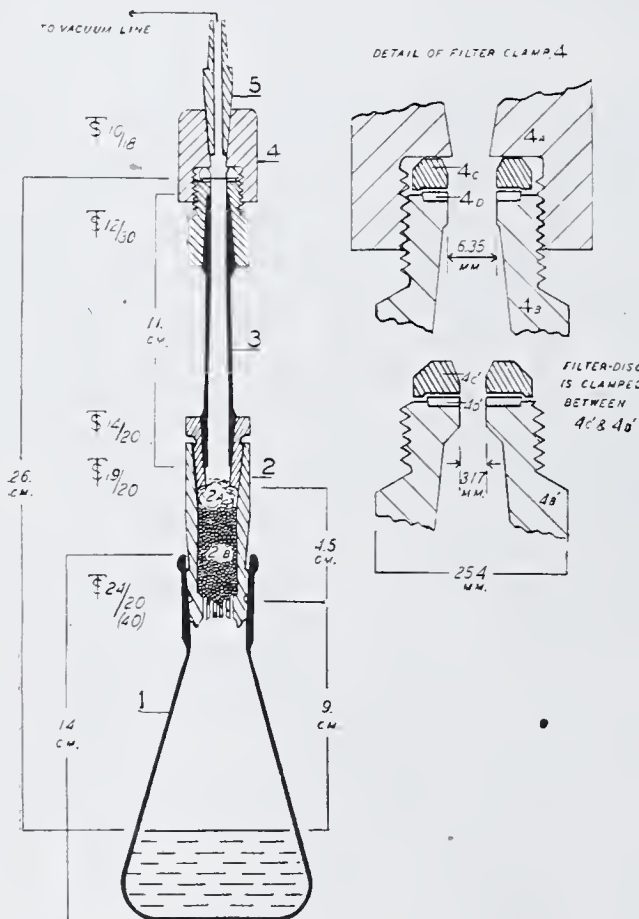


Figure 1. Diagram of Apparatus

1. Pyrex flask, 200-ml. capacity
2. Absorption tube, machined from methyl methacrylate resin
  - 2A. Pyrex wool
  - 2B. Pyrex, granular, 10- to 20-mesh, saturated with  $Pb(C_2H_3O_2)_2 \cdot 3H_2O$  solution
3. Pyrex delivery tube with ground-glass 14/20 and 12/30 joints
4. Filter clamp assembly (methyl methacrylate resin)
  - 4A. Upper member
  - 4B. Lower member with 6.35-mm. jet
  - 4B'. Lower member with 3.17-mm. jet
  - 4C, 4C'. Compression washers
  - 4D. Metal seat, central opening precision-reamed to 6.35 mm. and gold-plated
  - 4D'. Same as 4D, 3.17-mm. opening
5. Connecting tube for vacuum line (methyl methacrylate resin)



scrubber, 2. This part is taper-turned from methyl methacrylate resin, 2.5-cm. (1-inch) rod stock, with a 24/40 lower premity and relief groove to fit the flask (1, Figure 1), and its bottom is drilled symmetrically with 19 holes of 1.2-mm. diameter. Its cavity has an inside bore of 16 mm. in its lower (cylindrical) portion and is tapered 19/22 in its upper portion to receive a stopper which is taper-turned out of the same material 19/17 on the outside. This stopper is drilled and taper-bored 14/28 to receive the tube and stopper assembly. This forms a chamber to contain a 15-gram portion of granular Pyrex, 10/20 mesh, 2B, which is saturated with lead acetate solution and surrounded by a pledget of Pyrex wool, 2A, impregnated with the reagent, dried.

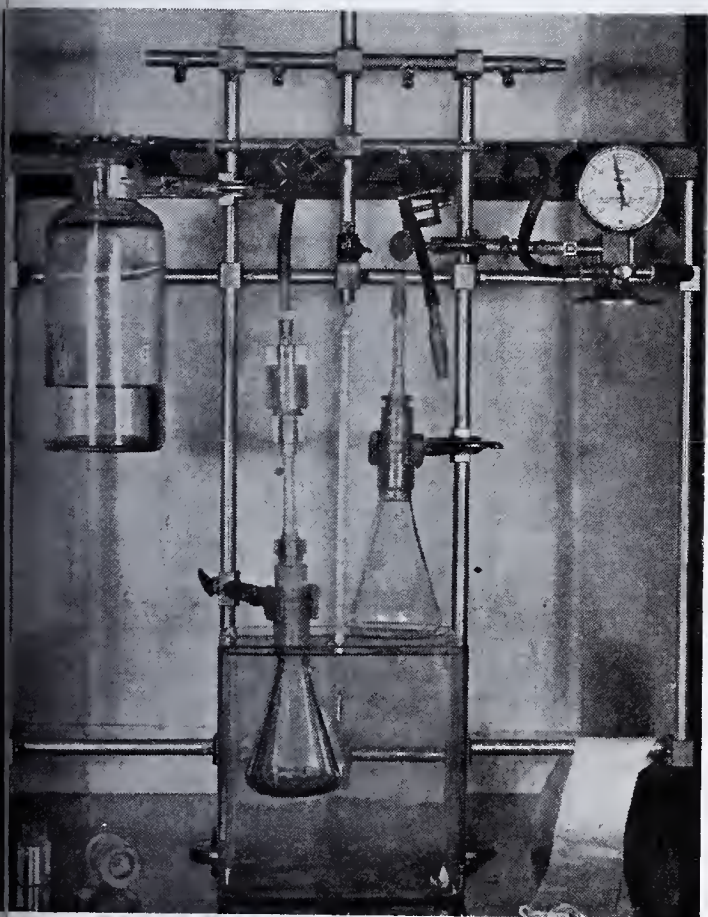


Figure 2. Apparatus

The Pyrex delivery tube, 3, has an upper 12/30 ground joint and a lower 14/20 ground joint to fit parts 4B and the stopper of respectively. Its internal diameter is approximately 8.5 mm. at the lower end and 6.5 mm. at the upper end; its length is 11

The filter clamp assembly consists of 2 members: the lower upper member 4B, machined from 2.5-cm. (1-inch) methyl methacrylate rod stock, threaded 3/4-inch, 16 threads per inch, Amer. Std. machine screw thread, drilled centrally and tapered 12/30 to fit upper joint of 3. Its upper surface is countersunk 0.1 cm. (0.040 inch) deep by 1.24-cm. (0.495-inch) diameter to receive a gold-plated silver washer 1.25-cm. (0.500-inch) diameter, 0.15 cm. (0.060 inch) thick. This washer (before plating) is bored and precision-reamed centrally to 6.35 mm. (0.25 inch) and given a slightly tapered outer edge for tight fit into 4B, thus forming a permanent precision-gaged gas for 6.35-mm. spot-filtration. It also provides a nonadherent tight seat for contact with the under surface of the mercuric chloride-impregnated filter disk, which is held in compression against it by 4C under clamping action of 4A (see detail, Figure 1). 4B' is an exactly similar lower clamp member provided with gas jet for 3.17-mm. (0.125-inch) diameter spot-filtration.

4C and 4C' are compression washers, precision-surfaced and machined from 1.9-cm. (0.75-inch) methyl methacrylate rod stock, 0.6-cm. (0.25 inch) outer diameter and drilled centrally 0.6 cm. (0.25 inch) and 0.125 inch, respectively, to correspond to 4B and 4B'. These compression washers are tapered at the outer edges to present a circular bearing surface 0.3 cm. (0.125 inch) wide for screw compression by 4A.

The upper clamping member, 4, 4A, is machined from 3-cm. (1.25-inch) methyl methacrylate rod stock and has a 3/4-inch  $\times$  16, Amer. Std. machine screw thread to fit 4B and 4B'. It serves for compressing washers 4C and 4C', thus effecting a marginal seal for the sensitized filter disk when seated upon the gas jet of the lower clamping member. It has a precision-surfaced bearing for contact with compression washers 4C and 4C' and is bored centrally with a 0.6-cm. (0.25-inch) hole and then taper-bored 10/18 to give outlet for gas through connection with 5.

The connecting tube to vacuum line, 5, is machined from 1.25-cm. (0.5-inch) methyl methacrylate rod stock by boring centrally with a 0.3-cm. (0.125-inch) hole and taper-turning on the outside 12/30 at the proximal end for connection with 4 and distally for connection with rubber pressure-tubing of the vacuum line.

The vacuum line may be actuated by a laboratory motor pump, or an ordinary water pump used for filtering may be attached, provided that the water pressure is adequate and fairly constant. The latter form of pump will, at best, not operate more than two filtration units simultaneously. In any case there should be a water trap in the line, as shown in Figure 2, and a convenient form of vacuum gage should be used, provided with an air-intake valve for control of pressure reduction in the system. During hot weather it is advisable to use a cooling jacket of moistened cotton stockinet (an infant's size no. 4 stocking with toe removed answers well); this should surround the delivery tube and clamping mechanism and be evaporated in the air current of an electric fan.

## METHOD

In planning a method for isolation and determination of arsenic as existing in organic substances in unknown combination, not only must the problem of cyclic compounds resistant to oxidation be met, but the problem of thermolability and unknown vapor pressures of component arsenic groups, which may volatilize under natural conditions, must be considered, especially in ultramicrodeterminations. In dealing with fresh animal tissues and with other kinds of unstable organic material, it may therefore be well to avoid the idea of "preparatory" treatment being a detached process, and to substitute the concept of a definite analytical partitioning of procedure, *ab initio*. The authors recommend two analytical steps in examining such material, both leading to a test solution suitable to Gutzeit reduction. The first is directed towards capturing any heat-sensitive arsenic component, in however minute concentration, by evaporation in a distilling system. The second step is to isolate the relatively "fixed" arsenic component which remains in the dehydrated residue, and which may be resistant to ordinary methods of oxidation, by submitting this residue to oxygen-bomb combustion. The two solutions which result from this partitioning may be separately tested for their arsenic content, or they may be combined for determination of "total arsenic" in the sample.

1. A measured sample of the material is taken in as fresh and natural condition as may be obtainable. For example, 2 cc. of circulating blood are withdrawn from the vein and are at once deposited through the needle of an aspirating syringe upon a small bundle of arsenic-free absorbent cotton suspended within a distilling flask ( $A_2$ , Figure 3) which is already connected with the vacuum-operated absorption train. On removing the needle from the side tubulature of flask  $A_2$ , this opening, 1, is screened with a cotton filtering plug (as shown at 1A in the larger flask,  $A_1$ ) to prevent the possible access of any dust or extraneous matter from the oxygen line, and is at once connected with a controlled supply of pure dry oxygen gas as obtained from liquid air. A minimum flow of this gas is now started while the flask is heated on a water or sand bath to 60° C. The flow of warmed oxygen gas, aided by suction of a vacuum pump at the end of the train, removes all volatile products and moisture from the preparation under a reduction of pressure which can be varied throughout the operation to suit the optimum speed of evaporation. The vapors are conducted from the flask through the acid-nebulizing jet, 1B, which sprays hydrochloric acid (1 to 1) into the mixing chamber, 2; here they are chlorinated and mixed with a cloud of ammonium chloride which is produced by ammonia gas derived from the reservoir, 3, containing ammonium hydroxide, which reagent, by adjustment of the rotating stem, 4, may be varied as to its surface area. This adjustment is to provide a visible cloud of



ammonium chloride in the combined vapors to serve as an indicator of the rate of absorption throughout units *C*, *C'*, and *D* of the train, all of which contain sulfuric acid (1 to 3).

The temperature in the distilling flask is maintained at 60° to 80° C. until there remains only a desiccated residue. In the case of 2-cc. samples of blood, this procedure requires about 30 minutes and somewhat longer for similar amounts of other animal and vegetable tissues. For larger (5- to 15-cc.) samples of liquids, such as urine and liquid culture media, a larger flask, *A*<sub>1</sub>, with reflux condenser is used, and evaporation is proportionately prolonged. The heat-labile components and most of the moisture having been evaporated and extracted in the absorption train, the vacuum line and oxygen supply are disconnected. All extracts, with washings from the train, are then made suitable to Gutzeit reduction (12% acid concentration) by dilution to a volume of about 100 cc. This constitutes a test solution representing the first or "volatile" fraction of the sample under analysis.

2. In the second analytical step, the dehydrated residue—consisting, in the case chosen, of dried blood on cotton—is promptly removed from flask *A*<sub>2</sub>, Figure 3, and placed in the cradle fixture, 13, of the oxygen bomb, *A*<sub>3</sub>, where its contained fuse wire, 4, is connected to the terminals, 10A and 11, of the ignition system in the bomb head, 1. The cylinder or combustion chamber, 2, supported by its clamping collar (not shown) is then raised to the closed position as indicated in *A*<sub>3</sub>, and there securely sealed by a massive clamping mechanism (not shown). The bomb is then charged with oxygen at 17.6 kg. per sq. cm. (250 pounds per square inch) pressure and the charge ignited by closing the ignition circuit. This process is essentially as described in a former report (4), but with certain improvements in apparatus (manufactured by the Parr Instrument Co., Moline, Ill.) of more recent development.

After the combustion, which is practically instantaneous, the gases and smoke are completely exhausted from the bomb through its outlet, 4, which is connected to the same absorption train, *B*, *C*, *C'*, *D*, as described in the first instance. In the case the ammonium ions which are given off from the ammonium hydroxide in the communicating reservoir, 3, of the nebulizing chamber, *B*, 2, combining with the spray of hydrochloric acid, serve primarily to effect an ammonium chloride cloud precipitation of all smoke particles within the globular portion of this chamber. Thence the combustion gases are conducted under action of the vacuum pump into the remaining units of the extraction train which have been provided with the same reagents used in the previous operation. When the exhaustion is completed, any residual gases in the bomb are washed through the train by a current of oxygen admitted through the bomb inlet, 3, which is still in connection with the oxygen supply. The resulting extracts, combined with bomb residues and washings from bomb and train, are similarly made up to 100 cc. in volume to form another test solution which represents the second analytical fraction, or relatively "fixed" arsenic, of the original sample. Both test solutions may be reduced separately or combined and determined as "total arsenic".

**ISOLATION AND DETERMINATION OF ARSENIC FROM TEST SOLUTIONS.** Whatever the previous analytical procedure may have been, the test material is assumed at this stage to be an acid aqueous solution.

The degree of acidity, if not known, should be determined and adjusted to approximately 12% hydrochloric acid, when brought to 100 cc. in volume. This test solution, containing the arsenic which is to be isolated as arsine and then quantitatively determined, is placed in the hydrogen generator flask (1, Figure

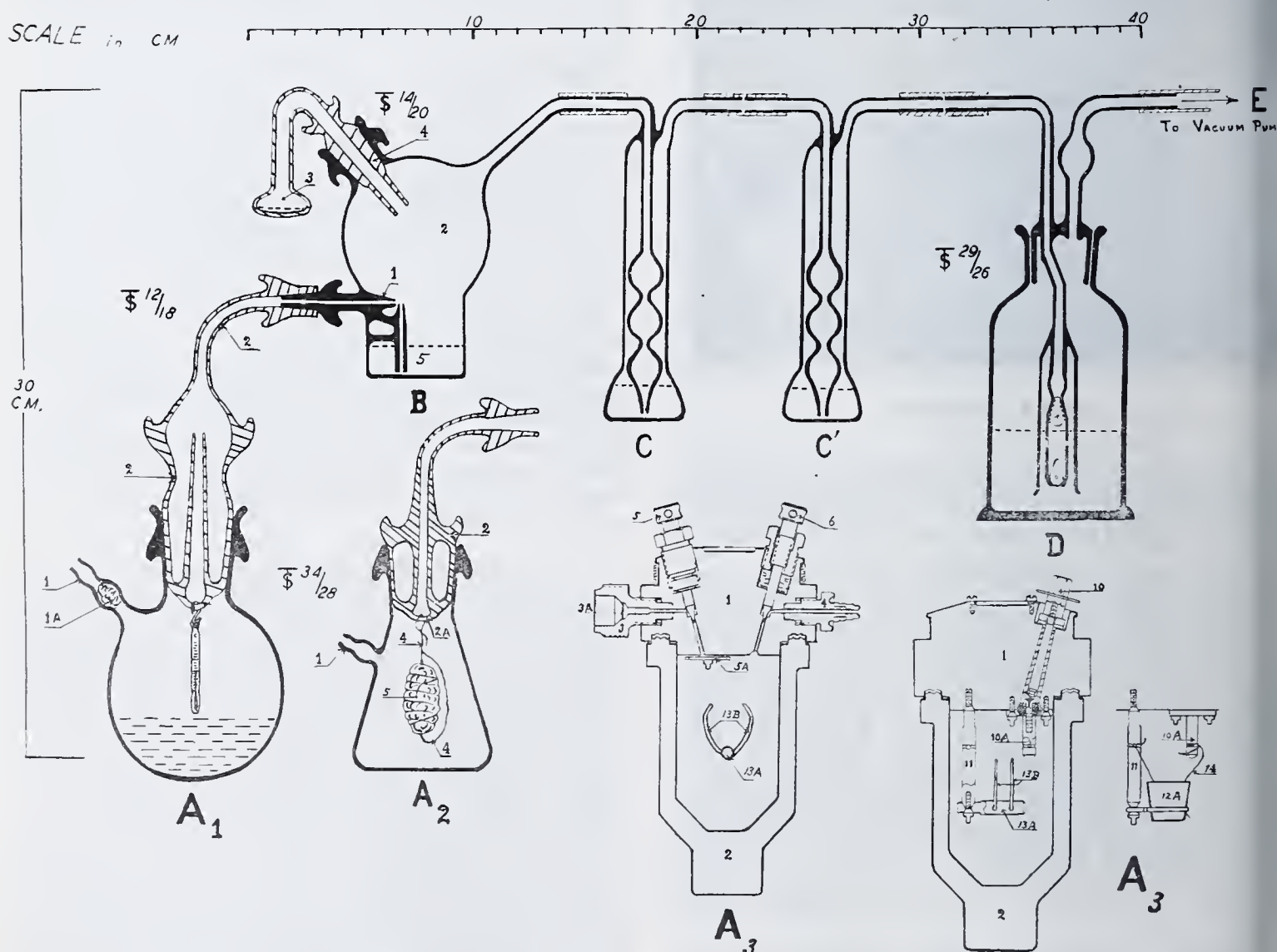
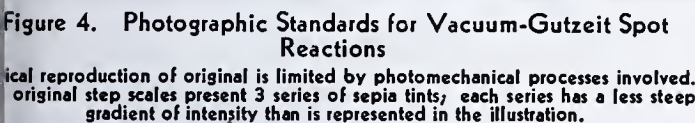


Figure 3. Apparatus for Separate Determination of Volatile and Fixed Arsenic in Small Quantities of Biological Material

*A*<sub>1</sub>, *A*<sub>2</sub>. Flasks for distilling and dehydrating material for analysis. *A*<sub>1</sub> is for 5- to 25-cc. samples of urine, liquid culture media, etc. *A*<sub>2</sub> is for smaller samples, 2 to 2.5 cc., or grams, of blood, fresh tissues, etc.  
*A*<sub>3</sub>, *A*<sub>3</sub>. Right sectional views of oxygen bomb used for flame combustion of dried residues  
*B*, *C*, *C'*, *D*, *E*. Vacuum extraction train used in connection with *A*<sub>1</sub>, *A*<sub>2</sub>, and *A*<sub>3</sub> for obtaining test solutions for ultramicrodetermination of arsenic by extraction of volatile products of evaporation or by extraction of smoke and gases from oxygen-bomb combustion of dry residue





the degree of arsenic reaction may be read directly by placing spot upon a white background and comparing its intensity in the shades of the photographic scale through windows which are punched in its banded portion. Anything which appears to be less than 0.04 microgram of arsenic in a 6.35-mm. (0.25-inch) test is considered a "blank". This corresponds to 0.01

In experiments with the addition of small amounts of sodium selenate to test samples of arsenical urine, they found that any concentration of selenium likely to occur—i.e., under 50 micrograms per 100 cc.—would be removed as selenium hydride along with hydrogen sulfide by action of the lead acetate scrubber. In the event of combined interferences, in cases of selenium or antimony poisoning complicated with arsenic poisoning of organic material, they have been prepared to work with relatively large samples and to remove phosphorus and antimony by distilling with 45% hydrobromic acid and an excess of bromine into the vacuum-extraction system, thus isolating arsenic with selenium in the distillate; then to separate selenium from arsenic by precipitating the selenium from the distillate with sulfur dioxide and hydroxylamine hydrochloride, according to the method of Robinson, Dudley, Williams, and Byers (21), leaving arsenic in the filtrate to be brought into a proper test solution for end determination by the present method, choosing an aliquot suitable to the ultramicro range.

The first essential for producing a photographic step-scale print for the evaluation of Gutzeit spot tests is a primary film trans-



parency presenting a banded scale of light-transmission densities, arranged in a progressive exponential series, and in convenient dimensions for contact printing—as, for example, showing 21 contiguous bands covering a strip of film 20 to 22.5 cm. (8 to 9 inches) long and of convenient width, as 1.375 inches or 35 mm.

Such a primary step-scale transparency may be made on an optical bench by successive steps of equal time-unit exposure to a constant source of light and at progressive distances from the light, the precisely measured distances constituting a definite logarithmic series of steps to produce a corresponding logarithmic series of densities in the exposed photographic film after development. A special machine, such as the Eastman sensitometer, Type IIb, devised for testing the characteristics of photographic emulsions, will accomplish the same purpose. The authors produced their first films for photographic standards by the optical bench method, but they do not recommend it, since it not only is very laborious, but requires much technical skill and experience and faultless technique to get satisfactory results. They later procured from the Eastman Kodak Co. a number of photographic "step-tablet" transparencies made by their sensitometer machine. These step tablets are now commercially available for use in 3-color process work. One of these transparencies which was accurately calibrated at the Physics Department Research Laboratories of the Eastman Kodak Co., Rochester, N. Y., served to produce the secondary negative film referred to in Figure 5, and from which the step-scale prints illustrated in Figure 4 were printed.

This secondary or master-negative film is required, because the steepness of gradation of the original film is too great to serve directly for making prints which will have the finely graduated steps that are required in the final product.

A negative is therefore made by contact-printing from the primary transparency upon an 8 × 10 film having a rapid, long-scale emulsion, such as "Defender, XF-Panchromatic" or "Eastman, Tri-X Pan AH", both of which emulsions possess a long straight-line portion in the low-development gradients of their characteristic (*D-log E*) curves. The aim here is to produce a negative step-scale film transparency as perfect as the original, but with its series of densities reduced in range while retaining the same number of steps as in the original—for example, from density 0.45 to density 2.41 in 21 steps as shown in Figure 5 (inset), a zone covered by only 14 steps of the original film.

This result is obtained by suitable exposure and development, so that the secondary negative, when calibrated by densitometer and plotted as a graph, will show a straight line similar to that of the original film, but with a lesser slope or gradient corresponding to a gamma of definitely shortened development. This task requires patience and considerable technical skill; but, once accomplished, there is no further difficulty and the resulting master negative is permanently useful for making contact prints on bromide paper to serve as standards for a variety of ranges of determination, as illustrated in Figures 4 and 5.

A suitable grade of bromide paper is required for making prints. It must be of pure white stock with a matte or rough surface. Its emulsion must be susceptible to full development without tendency to fog, and capable of giving a warm tone which will match the tinge of the ammonia-treated spot reactions. This is accomplished by adjusting the proportion of potassium bromide in the developer. "Defender, Velour Black, C-1", developed with "55D" formula diluted 33 to 50% to include addition of 10 to 20% of potassium bromide solution (10% by weight), has given good results. The time of exposure and development will vary slightly with different batches of paper. For instance, print B, Figure 4, was "VB-C-1" exposed 30 seconds at 85 cm. (34 inches) from a 60-watt Mazda lamp and developed in "55D" (12.5% potassium bromide solution added) at 65° F. for 50 seconds, the darkest band appearing at 22 seconds and the lightest at 49 seconds; print A, same paper and conditions, was exposed at 90 cm. (36 inches) from the lamp, and development was 28"/58"; print C was obtained by using a slightly "harder" grade of paper ("VB-C-2"). An acetic acid short-stop bath is used to stop development abruptly, and the print, after thorough rinsing in this, is fixed in two successive trays of plain "hypo" solution, 3 to 5 minutes in each tray with agitation. After a wash in running water for 20 to 30 minutes,

the print should be treated to eliminate any thiosulfate which cannot be removed by washing, since residual traces as small as 1 microgram per sq. cm. of paper, will, in course of time, produce slight fading in the bands of lesser density due to gradual "sulfiding" of the silver deposit. To correct this, a method of hypo elimination devised by Crabtree (9) is most reliable, a process which utilizes hydrogen peroxide and ammonium persulfate to oxidize every trace of thiosulfate for removal in a 5- to 10-minute period of final washing.

Even with these precautions, atmospheric conditions may cause degradation of the lesser densities, as, by hydrogen sulfide or sulfur dioxide in the air of industrial centers, or by salinity near the sea coast. A calibrated silver print should therefore be regarded as a delicate photometric standard and preserved carefully from the action of light, heat, and moisture; unless subjected to the sulfur-eliminative treatment recommended above, it should be recalibrated after 6 months. In any circumstances, a new standard print should be made from the master negative when a 12-month period has elapsed.

Prints are standardized by calibration against known amounts of volumetric arsenic solution as specified under Reagents. This is done by photometry, but expert visual calibration from known test spots, or from previously calibrated prints, will give

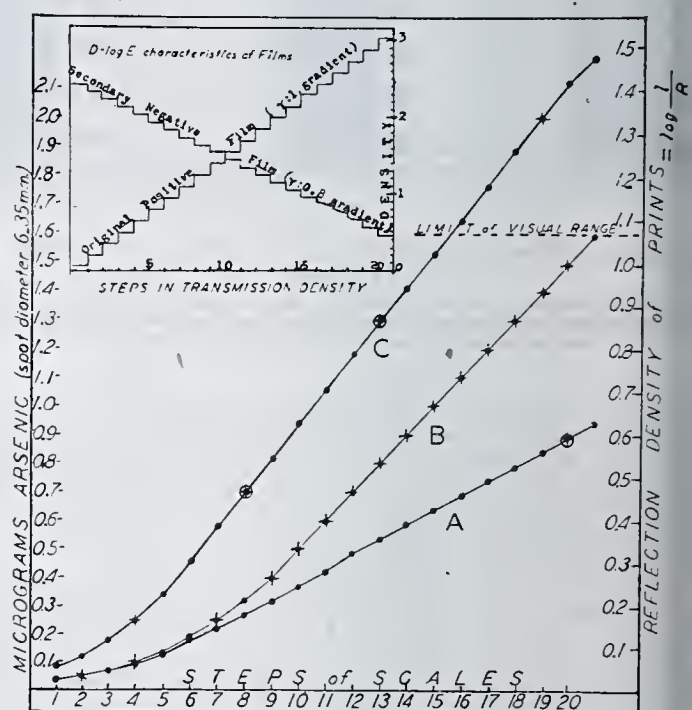


Figure 5. Reflection Densities of Photographic Scales

Step-scale prints A, B, and C with arsenic values coordinated to indicate system of calibration.

Dots are plotted against the right-hand ordinate of reflection density, expressed as  $\log \frac{I}{R}$ , where  $I$  is the intensity of light reflected from the blank paper base, free of deposit (whether Ag deposit of the print, or As-Hg deposit of the test reaction), and  $R$  the intensity of light reflected from the deposit. The abscissa measures the *D-log E* characteristics of the developed bromide emulsion of the prints, as printed through corresponding bands of the negative film shown in the insert. The left-hand ordinate is an arithmetic progression, in terms of micrograms of arsenic as contained in Gutzeit test spots 6.35 mm. in diameter—a unit test area selected to establish two datum levels of 1.00 and 0.10 microgram of As, amounts which in repeated photometric evaluations coincided very closely with 0.666 and 0.066, respectively, on the reflection density scale, as indicated at steps 15 and 4 of the B-print graph. Dots occurring alone at step intervals in the graphs represent photometer allocations of the corresponding bands of prints and refer to the right-hand ordinate.

Crosses represent definite amounts of arsenic added to test solutions, and are the theoretical basis of calibration as referred to the left-hand ordinate. Dots which closely coincide with crosses are the actual coordinating factors, plotted as calibrating factors by photometer readings of their reflection densities, and representing arsenic found, they are thus referable to both ordinates. Differences between crosses and nearly coincident dots, denote recovery data, or error of determination, and are summarized in the text as applying to different ranges. Dots marked with a ringed cross are duplications of primary calibrations on graph B. It is evident that this system of photographic standards for evaluation may be resolved to the formula:  $\frac{a(RD)}{0.666}$ , or,  $1.5a(RD) = \gamma As$ , where  $a$  is the unit area of 6.35 mm. diameter and  $RD$  the reflection density in terms of  $\log \frac{I}{R}$ .



ults which are surprisingly close to calibrations by galvanometer readings with a photoelectric cell. For photometric calibration the authors have employed the following apparatus:

A selenium photocell with a 20-mm. effective diameter, giving photocurrent of 150 microamperes and output of 25 microwatts (1 lumen). This is placed in an insulated adapter, or in a microphotometric slit ocular, at the eyepiece position of a microscope and connected with a mirror galvanometer of approximately 750-ohm resistance and a sensitivity of  $2.5 \times 10^{-9}$  ampere per mm., with the interposition of a compensating resistance circuit, so designed as to provide selective sensitivity with a stable zero point on the galvanometer scale under a wide variation of light intensities or field areas.

A microscope fitted with 24-mm. and 40-mm. single-lens objectives, and with tube length adjustable from 155 to 200 mm. (controlling exact size of field, a mechanical stage for multiple reduced field) readings, and a quick-shifting device for alternately positioning test spots and step-scale bands within the illuminated field of the instrument. (Precision of operation is assured by averaging or integrating fractional readings by means of a microphotometric slit ocular as described by Lange, 18.) An efficient type of electric microscope lamp and condenser, adjustable to an approximately  $45^\circ$  angle of incidence to prevent specular reflection, and capable of illuminating a field of 7 mm. or less in diameter. This lamp must be operated by a storage battery to ensure a nonfluctuating current and must produce a light of high intensity which is so steady as to cause no oscillation of the galvanometer light beam.

With such a system, the difference in photocurrent of reflections from unit areas of adjacent bands of a step scale, such as scale B in Figures 4 and 5, will give deflections of the light beam on the galvanometer scale of 3 to 15 mm., as controlled by sensitivity adjustment in the balanced circuit. Reactions from known amounts of arsenic or from unknown test solutions can be nearly matched with scale-band deflection readings and values interpolated according to the step deflection.

The precision of the combined vacuum-Gutzeit procedure and photometric reading method, when summarized from the recovery data of Figure 5, shows: (a) in the lower part, or "toe" portion, of curves B and C (from 0.04 to 0.40 microgram of arsenic), comprising 7 recoveries, an accuracy of 95.85%, with a mean deviation of  $\pm 0.04$  microgram; (b) in the range covered by the straight-line portion of the graphs (from 0.50 to 2.0 micrograms of arsenic), comprising 15 readings on 12 recoveries, an accuracy of 99.93%, with a mean deviation of  $\pm 0.0053$  microgram; (c) an over-all accuracy, for the entire range, of 96.3%, with a mean error of  $\pm 1.37\%$ . This applies to an exemplary set of findings and is somewhat better than the general average of  $\pm 1.5\%$ , which is claimed for the photometric application of the method. For routine visual evaluations in the optimum range of 0.04 to 0.90 microgram of arsenic, precision is estimated as within  $\pm 0.03$  microgram, and the visual error increases from  $\pm 0.06$  to  $\pm 0.07$  microgram in the higher range from 0.90 to the visual limit of 1.6 micrograms of arsenic, which implies a  $\pm 3\%$  error in the optimum range.

Only the bands of reflection density on the calibrated scales are the essential criteria for arsenic determination, and these represent ascending gradients of arsenic value strictly according to the area of the test spot. When such a step-scale print has been calibrated for the unit area of 6.35-mm. (0.250-inch) diameter, as shown in Figures 4 and 5, it follows mathematically that any step of the scale when referred to 3.17-mm. (0.125-inch) test spots, will represent just one fourth of the value attributable to the unit area. Thus, if an area be selected that is 2.5 times that of the unit area, or 10 times that of the 3.17-mm. area, its calculated area would be 79.167 sq. mm., its diameter 10.04 mm., and there would be established a triple ratio of 0.25:1:2.5 or 1:4:10, in scale value, according to spot area, with corresponding arsenic values holding throughout all shades of the scale.

## SUMMARY

A relatively rapid procedure and new apparatus are described for isolation under vacuum of small amounts of arsenic as arsenic trihydride. The method is especially applicable to very small samples of fresh biological material and other organic substances which demand an ultramicro range of determination. It employs the well-known zinc-acid reduction process, which is shortened in time of operation to 15 minutes and intensified in action without applying heat. This is accomplished by operation in a vacuum system at 0.25 to 0.5 atmosphere to produce an arsine-mercuric bromide reaction by spot filtration. The reaction is concentrated by jet-filtration through a precisely gaged area of the sensitized medium. The diameters of the jets producing the spot reactions are designated as 3.17 mm. (0.125 inch) and 6.35 mm. (0.250 inch), giving two orders of reaction areas in the ratio 1 to 4, and providing ranges of 0.01 to 0.40 and 0.04 to 1.50 micrograms of arsenic, respectively.

These Gutzeit spot reactions, after intensification with ammonia, are immediately evaluated by reference to a standardized photographic step-scale print having 20 bands of mathematically graded reflection densities, which have been photometrically calibrated against fresh spot tests from known amounts of arsenic. The method of calibrating these photographic standards by coordination with recovery data, and the photographic procedure for producing the prints and the permanent film negatives from which they are derived, are described. Apparatus comprising the vacuum-filtration system consists mainly of parts which are precisely machined from methyl methacrylate polymer.

Sensitivity claimed for the method in its present form is 0.01 microgram of elementary arsenic, which is visually distinguishable from a "blank". Accuracy claimed for the vacuum-Gutzeit method combined with evaluation by photographic standards is  $\pm 3\%$  by visual inspection in the range 0.02 to 0.90 microgram of arsenic and  $\pm 1.5\%$  by photometry in the extended range to 2.0 micrograms of arsenic. Larger or smaller filtration areas may be used for other ranges than those described.

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# Microchemical Identification of Demerol

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Demerol may be identified microchemically through formation of crystals with alkaloidal reagents. Doubly confirmatory test is available with single reagent, in conjunction with scratching of test drops. Tests with picric acid, lead iodide, sodium nitroprusside, potassium dichromate, and potassium iodide are described.

**A**LKALOIDS and alkaloidlike compounds, in general, combine with the so-called "alkaloidal reagents" to form insoluble complexes, many of which crystallize in unique and characteristic forms. Most of the natural alkaloids and many synthetic alkaloidlike compounds may be definitely identified by visual microscopic examination of these crystals. Several books and many articles describe or illustrate a number of those for which the specificity of the microcrystalline form has been established (1, 4, 5, 8 and others).

The usual procedure in making the microchemical tests is to add a drop of the reagent to a drop of a solution of the alkaloid on a glass slide; the test drop is then allowed to stand until crystal formation takes place. Rubbing or stirring the test drop to induce or hasten crystallization is, in general, considered to be objectionable. Stephenson (6) states that crystals formed by stirring are likely to be less characteristic than those formed more slowly, and recommends that stirring be avoided to prevent the formation of abnormal crystal forms. Scratching is sometimes

recommended to induce crystallization (2), but no reference has been found in which scratching is used to influence the course of the crystallization.

Demerol (ethyl-1-methyl-4-phenylpiperidine-4-carboxylate, 3) may be readily identified microchemically. Its reaction with 28 of the common alkaloidal reagents was studied. Of these, 13 combined to form crystals which are very well formed and suitable for purposes of identification; 7 other formed crystals which are either because of low sensitivity or because of atypical crystal formation, are less suited for the purpose; 5 formed only amorphous precipitates; and 5 failed to form any precipitate.

The effect of scratching the test drops of Demerol and picric acid, sodium nitroprusside, or potassium iodide is very striking. Well-formed individual crystals are obtained which are entirely different in appearance from those formed in the undisturbed test drop. They do not appear in any way to be merely distorted or abnormal forms of the latter but are crystallized in distinct and unique forms which are very well suited for microscopic examination. Advantage may be taken of this circumstance in having a doubly confirmatory test for Demerol: the formation of two distinct and individual crystal types with a single reagent, in conjunction with the unique influence of scratching in effecting the formation of this dual pattern of crystal types, furnishes a cross-combination of data ensuring the reliability of the identification.

No information is available at present as to the effectiveness of the scratching technique in the identification of other alkaloids with the various alkaloidal reagents. Preliminary work indicates that fruitful results might be obtained; a study of the subject would be of decided interest.

In making the tests, the usual procedure was employed. To one drop (0.03 ml.) of an aqueous solution of Demerol hydrochloride was added an equal drop of the reagent. The resultant crystals, formed either on standing

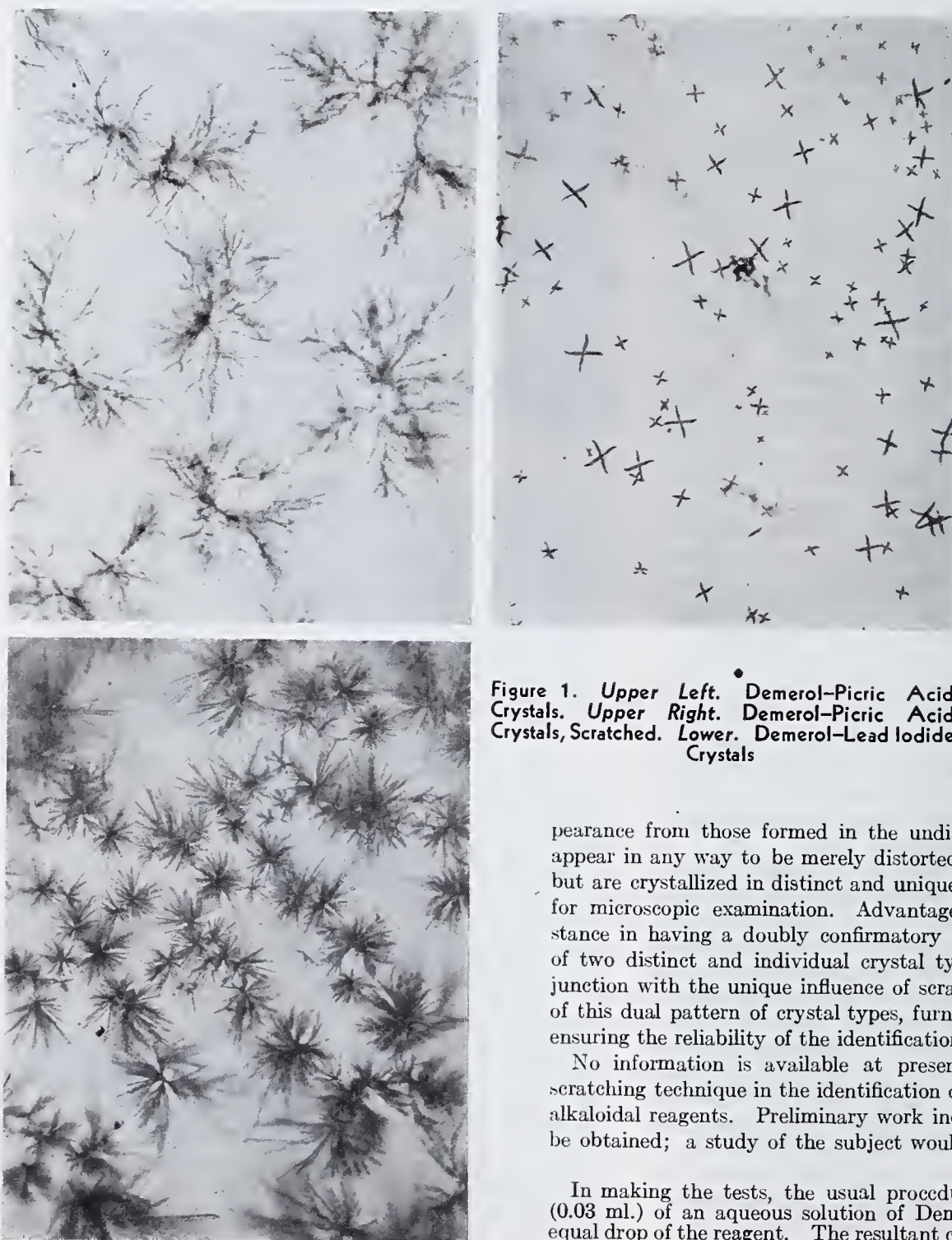


Figure 1. Upper Left. Demerol-Picric Acid Crystals. Upper Right. Demerol-Picric Acid Crystals, Scratched. Lower. Demerol-Lead Iodide Crystals



scratching with a glass rod, were examined under a magnification of 90X. Best results, in regard to facility of examination of the crystals, were obtained if the concentration of the Demerol solutions was such that not more than a slight initial precipitate formed upon addition of the reagent. Hydrochloric acid, 4N, may be used instead of water as solvent for the Demerol, except in the case of picric acid. Here the effect of the acid prevents the formation of the characteristic X-shaped crystals.

Crystalline complexes were obtained from the reaction of Demerol with picric acid, potassium iodide, sodium nitroprusside, potassium iodide, potassium dichromate, potassium chromate, chromic acid (the latter three in hydrochloric acid solution), potassium cyanide, potassium ferricyanide, platinic chloride, mercuric chloride, and palladium chloride. Amorphous precipitates were obtained with gold chloride, Wagner's reagent, Marme's reagent, Mayer's reagent, phosphotungstic acid, phosphomolybdic acid, silicotungstic acid, zinc chloriodide, sodium phosphate, sodium cobaltinitrite, and picrolonic acid. No precipitate was obtained with ferric chloride, zinc chloride, ammonium thiocyanate, saccharine, or potassium permanganate.

#### REAGENTS AND CRYSTALS BEST SUITED FOR IDENTIFICATION

PICRIC ACID, saturated aqueous solution.

This reagent is very sensitive. Best results are obtained with concentrations of Demerol of 0.1% or less. An amorphous precipitate forms, which is transformed on standing to rosettes, the arms of which are very fine, wavy filaments (Figure 1, upper

left). If the reaction drop is scratched immediately after addition of the solution, X-shaped crystals (Figure 1, upper right). At higher concentrations of Demerol, both forms of crystals may be found in the same test drop after scratching.

If the Demerol is dissolved in 0.1 N hydrochloric acid instead of water, no X-shaped crystals will form, even on scratching. Instead, there are formed, in addition to rosettes, long, very fine, needle-like crystals. Addition of a very small amount of sodium bicarbonate to the Demerol solution promotes the formation of X's; if

large amounts of sodium bicarbonate are used, some crystals of this type will form even in the undisturbed drop.

POTASSIUM IODIDE, prepared by the method of Wagenaar (?). Add to a 1 to 3% aqueous potassium acetate solution a drop of methyl red indicator, then add picric acid until the yellow color changes to light brown. Saturate with potassium iodide while warming gently, cool, and filter.

With a 0.1% solution of Demerol, an amorphous precipitate forms, changing to rosettes, the arms of which broaden on standing to form long flat plates (Figure 1, lower left). The rosettes lie in a horizontal plane, and overlie other rosettes lying in different planes.

If the test drop is scratched, short flat rods which look like the arms of the rosettes form along the scratch proper, while throughout the drop the types of rosettes as in the undisturbed test drop crystallize quickly.

POTASSIUM NITROPRUSSIDE, 5% aqueous solution.

With a 1% solution of Demerol, an amorphous precipitate forms, changing to long coarse bladelike plates, both individual and twinned (Figure 2, upper left). With solutions of lower concentration, no amorphous precipitate forms initially; the crystals slowly form from the edge of the drop.

If the test drop is scratched, small very distinct hexagonal prisms are formed (Figure 2, upper right). Concentrations of 0.2 to 1% Demerol are

suitable. In accord with the orientation of the prisms, the hexagons may or may not appear equilateral. The relative lengths of the sides may be such that the crystals appear rhomboidal or, sometimes, diamond-shaped.

POTASSIUM DICHROMATE-HYDROCHLORIC ACID. A 5% solution of potassium dichromate in a mixture of equal parts of concentrated hydrochloric acid and water.

An amorphous precipitate forms, changing to a dense mat of long yellow needles, often in sheaves (Figure 2, lower left). With concentrations of Demerol under 0.2%, no amorphous precipitate is formed; direct crystallization takes place slowly. Scratching hastens the crystallization, without affecting the crystal form.

Similar crystals are formed with potassium chromate or chromic acid in hydrochloric acid solution. Neutral aqueous solutions of the three reagents produce only amorphous precipitates.

POTASSIUM IODIDE, 20% to saturated aqueous solutions.

The sensitivity of this reagent varies with its concentration. A 20% solution is sensitive to a 0.2% Demerol solution, while sensitivity to below 0.05% Demerol solutions may be obtained by saturating a test drop of the latter with crystalline potassium iodide.



Figure 2. Upper Left. Demerol-Sodium Nitroprusside Crystals. Upper Right. Demerol-Sodium Nitroprusside Crystals, Scratched. Lower. Demerol-Potassium Dichromate Crystals







Figure 3.  
Left. Demerol-Potassium Iodide Crystals.  
Right. Demerol-Potassium Iodide Crystals, Scratched.

If the test drop is allowed to stand, very long needles, both individual and in sheaves, form (Figure 3, left). Crystal formation in the undisturbed drop is slow with low concentrations of Demerol and of potassium iodide.

If the test drop is scratched, the resultant crystals are short blunt rods, sometimes twinned (Figure 3, right). These crystals are characterized by their high refractivity and by their pronounced uniformity in length.

It is probable that crystals well suited for purposes of identification would be obtained with other alkaloidal reagents. The

reagents used, however, are common, and the above descriptions provide adequate data for the unequivocal identification of Demerol.

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## Semimicrosaponification of Esters

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A general semimicroprocedure for the saponification of esters, based on saponification with 2 *N* sodium hydroxide in a closed system, is described. The technique is readily adapted to routine analysis. A study of steric hindrance has given a measure of the limitations of the method and permitted the development of relatively mild conditions for a wide variety of esters, thereby minimizing the interference of other active organic functional groups.

THE saponification of esters as an analytical method has been studied primarily in the chemistry of fats and waxes (1, 5, 6). These drastic techniques, designed for the analysis of difficultly hydrolyzed esters, are not required for a large number of the carboxylic esters. Redeman and Lucas (4) modified the general analysis of esters by employing a closed system at temperatures ranging from 70° to 130° C. Bryant and Smith (2), while studying the effect of structure on saponification, developed a simplified procedure for the analysis of esters of widely varying structure. In their method, saponification is carried out in a closed flask using an excess of 2 *N* sodium hydroxide in 90% methanol.

This basic saponification medium has been retained in the semimicrotechnique. When applied to routine analysis, it has effected a considerable saving in reagents, amount of sample used and space required for apparatus. In the present paper, also, a

series of esters has been studied in order to determine conditions sufficiently mild to effect quantitative hydrolysis of the esters while minimizing the interference of aldehydes and ketones. The investigation included a study of relative activity of the esters at 60° C. in an alcoholic environment and at room temperature in 50% aqueous medium.

The presence of alkyl side chains near the ester groups is known to retard saponification. At the higher temperature  $\alpha$ -alkyl hindrance in the acid radical was particularly marked between the  $\alpha$ -methyl-substituted butyl and amyl esters. At room temperature this effect was noticeable even in the simplest esters.

#### EXPERIMENTAL

**REAGENTS.** Alcoholic 2 *N* sodium hydroxide is prepared by dissolving 80 grams of c.p. sodium hydroxide and 100 ml. of distilled water in sufficient Du Pont synthetic methanol to make 1 liter of solution.

Standard 0.2 *N* hydrochloric acid is prepared by diluting 19 ml. of concentrated hydrochloric acid to 1 liter with distilled water.

**APPARATUS.** A precise vacuum filling pipet (Figure 1) is used to deliver reproducible volumes of the 2 *N* caustic, which is stored in the 1-liter reservoir. The apparatus is protected with soda lime at all outlets.

The 10-ml. buret (Figure 2) is used to deliver accurately the standardized acid during back-titration. (These items were made



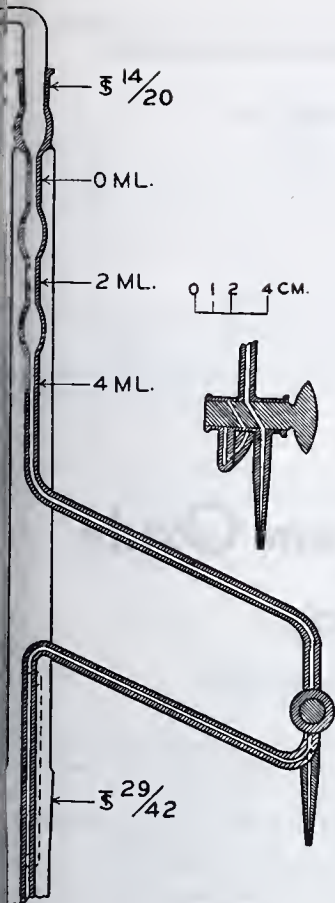


Figure 1. Delivery Pipet

ur H. Thomas Co. 60-cm. (24-inch) clamp-bars and No. G, size 3C, micro-spring-grip clamps, one bath will accom- te up to twenty 25-ml. or 50-ml. flasks, simultaneously.

ANALYTICAL PROCEDURE. A 2-ml. portion of the 2 *N* caustic ion is delivered from the vacuum filling delivery pipet into -ml. glass-stoppered volumetric flask. Then the sample, uining up to 2 milliequivalents of ester, is added or weighed the flask. In the general analysis the final concentration of m hydroxide should not be less than 1 *N*. This limits to 2 he total volume of sample or sample plus diluent. The together with a blank, is placed in a water bath at 60° ±

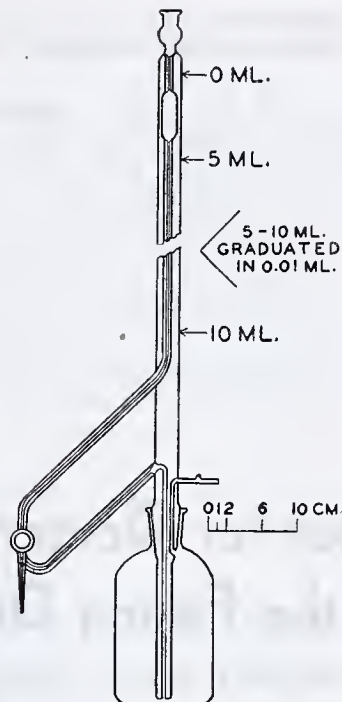


Figure 2. Microburet

to the authors' specifications by Eck and Krebs, 131 West 24th St., New York, N. Y.)

A convenient water bath (Figure 3), initially described in an earlier publica- tion (7), has been converted to a thermostatically controlled unit. Equipped with

Table II. Saponification of Esters in 30 Minutes at Room Temperature

Ester	Per Cent Saponified	
	2 <i>N</i> NaOH in 90% CH <sub>3</sub> OH	2 <i>N</i> aqueous NaOH
Methylacetate	91.5	100.0
Ethylacetate	94.0	100.0
Monoacetin	...	100.0
Isopropylacetate	91.0	99.5
Diacetin	...	100.0
Ethylpropionate	91.5	100.0
Triacetin	...	100.0
Isobutylacetate	95.0	100.0
<i>tert</i> -Butylacetate	...	76.0
Ethyl- <i>n</i> -butyrate	...	99.5
Ethylisobutyrate	69.5	100.0
Methyl- <i>n</i> -valerate	...	100.0
Methyl- <i>n</i> -caproate	...	84.5
Ethyl-2-methylbutanoate	...	50.0
Methyl-2-methylpentanoate	...	40.0
Ethyltiglate	...	98.0
Isobutyl- <i>n</i> -butyrate	...	34.5
Isobutylisobutyrate	...	50.0
Ethylethylmalonate	...	92.0
Ethyladipate	...	100.0
<i>n</i> -Amyl- <i>n</i> -caproate	...	5.0
Cyclohexylacetate	...	100.0
Cyclohexylisobutyrate	...	6.0
Ethylphenylacetate	...	100.0
<i>n</i> -Butylphthalate	...	6.0
Benzylbenzoate	...	7.5

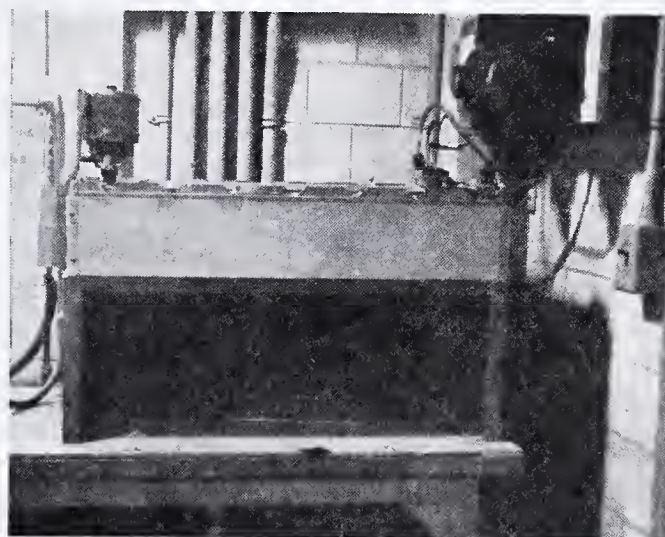


Figure 3. Constant-Temperature Water Bath

Table I. Analytical Data for Esters

Ester	No. of Determinations	Found, Weight %	
		Semimicro	Macro
ylacetate <sup>a</sup>	2	99.0 ± 0.0	...
acetate <sup>b</sup>	2	98.7 ± 0.2	...
acetin <sup>c</sup>	2	105.6 ± 0.3	...
opylacetate	2	98.8 ± 0.0	...
tin <sup>c</sup>	2	92.3 ± 0.2	...
propionate	6	99.7 ± 0.2	99.9
etin	2	99.2 ± 0.1	...
ylacetate	10	98.8 ± 0.2	98.7
ylacetate	2	97.0 ± 0.1	...
tylacetate <sup>d</sup>	2	98.3 ± 0.2	...
- <i>n</i> -butyrate	2	100.2 ± 0.0	...
sobutyrate	2	97.9 ± 0.2	...
l- <i>n</i> -valerate	4	98.8 ± 0.2	99.0
l- <i>n</i> -caproate	2	98.8 ± 0.2	...
l-2-methylpentanoate <sup>e</sup>	2	67.3 ± 0.0	...
iglate <sup>c</sup>	2	100.0 ± 0.0	...
2-methylbutanoate <sup>e</sup>	2	92.3 ± 0.0	...
yl- <i>n</i> -butyrate	4	98.5 ± 0.2	98.5
ylisobutyrate	2	99.7 ± 0.1	...
adipate	6	99.2 ± 0.1	99.2
ethylmalonate	2	92.8 ± 0.1	...
l- <i>n</i> -caproate	2	93.2 ± 0.2	...
yladipate <sup>c</sup>	4	100.2 ± 0.1	100.2
exylacetate	2	100.2 ± 0.2	...
exylisobutyrate <sup>c</sup>	4	97.9 ± 0.2	...
exyladipate	4	99.9 ± 0.2	100.0
phenylacetate <sup>f</sup>	2	99.7 ± 0.1	...
ylphthalate	2	98.5 ± 0.0	...
lbenzoate	4	100.1 ± 0.1	...

arbitrarily and Carbon Chemical. <sup>b</sup> J. T. Baker. <sup>c</sup> Eimer & Amend. <sup>d</sup> Prepared from *tert*-butanol and acetyl chloride in presence of pyridine. <sup>e</sup> Prepared from corresponding acid and alcohol. <sup>f</sup> Newport Chemicals. All others Eastman chemicals.

1° C., loosening the stopper momentarily to allow for expansion of included air. Then the flask is stoppered firmly, heated for 30 minutes, removed from the bath, and cooled in ice water. Excess alkali is determined by back-titration with the standardized 0.2 *N* hydrochloric acid to the phenolphthalein end point.

## RESULTS

Analytical results obtained on twenty-nine widely different esters are given in Table I, together with some comparative macro values (2). Except where noted the trade products were used without further purification. In general, the precision and accuracy are each about ±0.2%.

Interference due to α-alkyl substitution was noticed first with ethyl-2-methyl butanoate and ethylethylmalonate. However, the next higher homolog, methyl-2-methylpentanoate, reacted only to the extent of 67%, a decrease of about 25%. By increasing the temperature to 100° C. and heating for 2 hours the ethyl-ethylmalonate and ethyl-2-methylbutanoate were saponified quantitatively, while the methyl-2-methylpentanoate was 95% complete.

The presence of water in the sodium hydroxide solution is desirable for saponification in alcoholic reagent (3). In the general analysis, however, water in excess of 10% decreases the solvent



action of the methanol. These factors were verified during 30-minute room temperature studies, where the ester in methanol solution was added in 2-ml. portions either to 2 ml. of 2 *N* sodium hydroxide in 90% methanol or to 2 ml. of 2 *N* aqueous caustic. In the one case this represented essentially alcoholic environment and in the other about a 50% aqueous medium. Results obtained on several esters are given in Table II.

A comparison of results from the two reagents definitely proves the beneficial effect of water. In the alcoholic environment saponification was incomplete, even with the simplest esters. In the aqueous alcoholic medium, however, saponification was complete for most of the normal lower esters. *tert*-Butyl acetate showed the first marked evidence of side-chain interference in the

aqueous medium, while methyl-*n*-caproate represented the normal ester not completely soluble in the mixture.

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PRESENTED in part before the Division of Analytical and Micro Chemistry at the 106th Meeting of the AMERICAN CHEMICAL SOCIETY, Pittsburgh, Pa., 1934.

## Modifications of Apparatus for Deuterium Oxide Determination by the Falling Drop

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THIS note outlines modifications of the apparatus described by Keston, Rittenberg, and Schoenheimer (2) which permit greater speed of deuterium oxide analysis.

Two distillation trains are used (see diagram), the components of which are more readily cleaned and more interchangeable than those of Keston *et al.* The distillation trains are supported rigidly only at positions *i*. Where many determinations are to be made, the following glassware will be found sufficient: 10 tubes *a*; 3 each of tubes *b* and *c*; 2 each of tubes *d* and *g*; 14 plugs *e*; and 6 weighing bottles *f*. Tubes *g* with weighing bottles can be attached to tube *a*<sub>2</sub> in place of the plug when the sample contains no organic material and combustion is unnecessary. The outlet of the combustion furnace is adapted to fit the *a* tubes. The sample, with barium carbonate, is boiled for a few moments before the pressure is reduced, to eliminate bumping. Calcium oxide is used in tube *b* in place of potassium hydroxide and chromic oxide to eliminate replaceable H<sup>+</sup>. As condensing medium an ice-saturated calcium chloride bath is convenient, inexpensive, and entirely satisfactory.

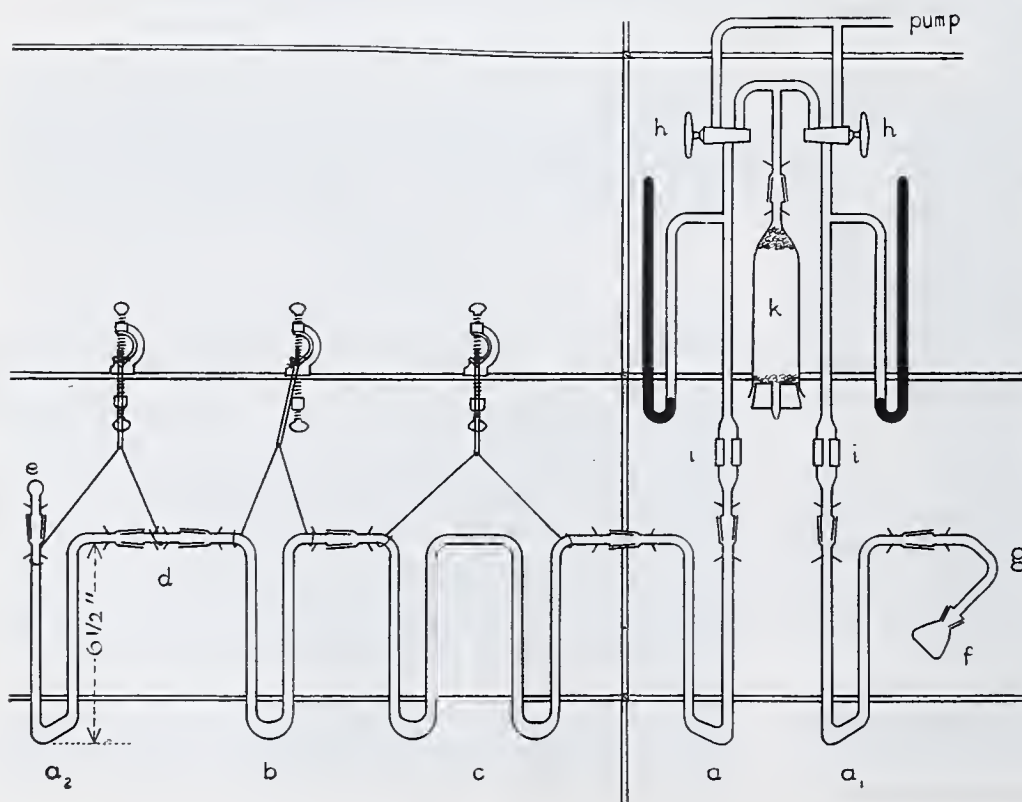
<sup>1</sup> Present address, Equipment Laboratory, Engineering Division, Wright Field, Dayton, Ohio.

An accurate, relatively simple, and easy to use microapparatus can be made from a microscope mount and a hypodermic syringe. Its operation is entirely mechanical. The outlet of a 1-cc. tuberculin syringe is attached rigidly to one end of an appropriately bent 0.5-mm. capillary; the other end of the capillary is drawn to a small tip. The syringe barrel and capillary are mounted firmly on the coarse-adjustment housing of the microscope mount in such a way that motion of the fine-adjustment housing is not interfered with. A spring is slipped over the lubricating plunger of the syringe before it is inserted into the barrel. The plunger is actuated by a precisely threaded pin (from an inexpensive micrometer caliper, for example). The threaded sleeve in which this pin turns is attached to the fine-adjustment housing. Two stops allow the pin to be turned through only part of a revolution. The capillary and syringe are filled, under vacuum, with mercury. The coarse adjustment of the microscope mount raises and lowers the entire pipet; the fine adjustment is used for focusing and flushing the capillary; rotation of the threaded pin through its prescribed limits delivers the droplet. The capillary may be provided with a removable tip for easy cleaning, and with a mercury reservoir.

Extremely vigorous stirring of the constant-temperature bath renders the maintenance of the required constancy relatively easy; such stirring may be achieved with little vibration with the impeller described by Hemingway and Shelley. As a thermoregulator, copper tubing filled with 1,2-dibromomethane gives excellent results; 1,2-dibromomethane has a low specific heat, high thermal conductivity, and high expansion coefficient, so that it is about as effective as the liquids usually used in thermoregulators. Ordinary cement cannot be used with this substance to attach the copper tube to the thermoregulator head; it should be sealed directly to the copper, or the tubing can be inserted into a copper Wood's metal surrounding the copper tube. The heater element used is an enameled copper wire connected to a secondary (10 volts, 12 amperes) transformer; this element has almost no heat lag. A mercury, constant-level overflow column is used to maintain a constant flow of cooling water through the bath.

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# Improved Apparatus for Solubility Determination or for Small-Scale Recrystallization

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VERAL types of apparatus useful in small-scale recrystallization, where centrifuge filtration is employed, were described in a previous publication (1). Since then, a number of modifications have been made in the design and, in view of the potential use of such devices, a description of the improvements would appear advisable.

For routine fractional recrystallization, the apparatus shown in Figure 1 is easily constructed, and is considerably superior to the previously described.

It is an ordinary test tube, 100 mm. long and 12 mm. wide, made from a smaller, thin-walled test tube 70 or 80 mm. long, approximately 8 mm. wide, and weighing between 2 and 3 grams. The upper half is widened by heating in the flame to just the melting point and slowly pushing it over a hot carbon rod a little over 8 mm. in diameter. By steadily turning the tube on the rod, a very uniform shoulder having an angle of about 45° can be obtained. Another test tube of the same size will then just pass through the enlarged part. *C* is made by heating a test tube 8 mm. in diameter with a firmly round bottom at a point about 15 mm. from the end and allowing the glass to collapse. When enough solid glass has collected at this point, it can be drawn out to form a solid rod 2.5 mm. in diameter, as shown in Figure 1, approximately 60 mm. long. The lower end of the rod can be enlarged as shown, by the use of a flat carbon while the tip is molten. This gives the rod more strength so that it can better stand the pressure during centrifuging. The combined weight of *B* and *C* should be of the order of 4 to 6 grams. *D* is a larger test tube which is fitted with a rubber stopper and has a hole in the bottom at *E*.

In actual practice, the material to be recrystallized is placed in the modified test tube, *B*, and filtration is achieved with a minimum volume of solvent. The apparatus is then assembled in the position the reverse of that shown in Figure 1, cooled to the optimum temperature for crystallization. After crystallization, it is inverted to the position shown and centrifuged. (A speed of approximately 1500 in a No. 2 International centrifuge has been found to give clear-cut filtration without breakage of the equipment.) The crystals are caught at the point of enlargement of test tube *B*, since there is just enough clearance between *C* and the shoulder of the enlargement of *B* to allow the liquid but not the crystals to pass through. For fine crystals, *C* may be ground a little more accurately on the shoulder of the enlargement with rough Carborundum, as described in (1). After filtration, the inner test tube, *A*, is first removed and held at an angle of about 45°. *B* and *C* can then be removed as one piece by grasping the enlarged end of *B*, since in this position *C* will bind in the larger part of *B* and will not fall out.

It is convenient to have *B* and *C* tared together, so that the weight lost during crystallization, the wet weight, etc., can be obtained. During filtration, *C* can serve as a stopper to prevent the entrance of extraneous material during storage or during further crystallizations and manipulations. When properly seated by centrifuging, the unit is entirely adaptable to solubility microdeterminations, as carried out by Ingber and Bergmann (2) and more recently in a greatly improved way by Moore and Stein (3).

A slight modification in the design adapts the apparatus for use with much larger amounts of material. This is done by enlarging the crystallization part of *B* by blowing it larger from the same test tube, as shown in Figure 2, or otherwise making a very thin-walled bulb. The bulb or crystallization part in this case must be very thin, so as not to increase the total weight of the crystallization vessel and the filter stick much beyond 6 grams. Bulbs holding from 15 to 20 ml. have been constructed from this weight of glass and successfully used by starting the centrifuge a little more slowly. This volume permits recrystallization from 10 to 15 ml. of solvent.

For small-scale work, sintered filters are unquestionably useful, both for suction and centrifuge filtration, but are not always easy to clean, and this gives rise to a reasonable distrust of their reliability. They also retain appreciable amounts of mother liquor, and complete removal of the crystals from the rough surface without scratching off glass particles at the same time is generally difficult. An attempt to devise an all-glass filtration apparatus free from these objections has met with success in the filter shown in Figure 3, which is an improvement over the one previously described (1) (Figure 2).

*A* is a glass funnel of the shape indicated. The narrow part of the funnel is made from glass tubing approximately 5 mm. in inside diameter, and is approximately 15 mm. long. *B* is made by stamping out a molten glass rod or tubing with the appropriate carbon surface, so that it is approximately the shape shown and then grinding it to fit more accurately. The narrow part of funnel *A* acts as a sleeve to hold *C* in position, so that the two surfaces at *C* may be ground with 120-mesh Carborundum just to the point where the funnel, when in position, will completely remove fine bone black from an aqueous suspension.

Such filters, properly prepared, give surprisingly rapid filtration when the ground surface of *B* is not too long. Therefore, the ground edge of *B* at the point *C* is rather thin (1 mm. or less). They are effective even with the finest bone black. They may readily be taken apart for cleaning and are free from the objections of sintered filters. They are particularly useful for micro work and allow filters of very small diameter to be constructed. The principle involved is fundamentally the same as that with sintered filters, except that here the walls of the pores which effect filtration may be taken apart for cleaning.

For solubility determinations, the authors have modified the excellent apparatus of Moore and Stein (3), as shown in Figure 4, for use with this type of filter.

Tube *A* is exactly as described (3), except that a ground-glass stopper is substituted for rubber. The stopper is hollow and has a wide, flat top, so that the inverted tube will stand on the stopper during the necessary manipulation. Two small indentations are on opposite edges of the flattened top for rubber bands to hold tube in the equilibrating apparatus. The inside surface of the stopper has a button on it to hold the flexible wire

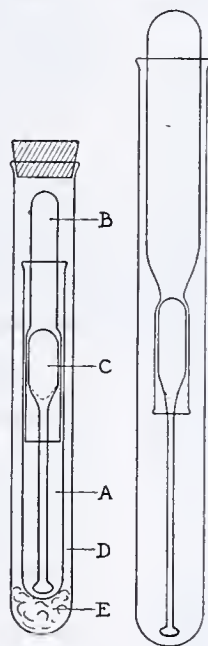


Fig. 1



Fig. 2

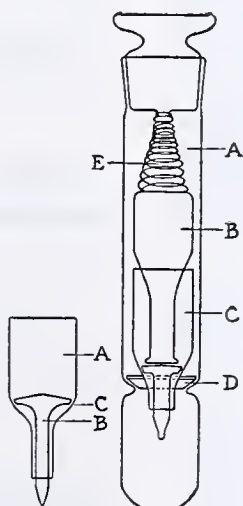


Fig. 3

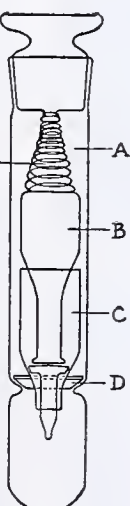


Fig. 4



spring, *E*, which in turn holds the filtration apparatus and filter stick in place. Flask *B* is of very thin glass, for its rests against the filter stick to hold it in place and a heavy flask would break during centrifugation. Filter *C* is as described above, except that the lower part is as short as possible. It rests on a soft tin collar, *D*.

Using this assembly, accurate solubility determinations may

be made with organic solvents or corrosive solvents, since no ber is present to absorb any portion of the solvent.

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## Semimicrodetermination of Arsenic in Insecticides

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THE procedure presented below represents an extension to insecticidal arsenicals of the method recently described (4) for the semimicrodetermination of arsenic in organic compounds, the arsenic being precipitated as element by action of hypophosphorous acid and determined iodometrically with the aid of Koppeschaar's bromide-bromate solution. This method is believed to be applicable to any properly prepared solution of arsenic free from interfering substances, such as organic material or metals precipitable by hypophosphorous acid. It was found that acid-soluble arsenicals (Paris green, lead arsenate, calcium arsenate) could be analyzed thus, following dissolution of the samples in aqueous hydrochloric acid.

In presence of organic material, a preliminary decomposition similar to that described for the analysis of organic arsenicals (2, 4) may be necessary. This was the case with the commercial insecticide currently marketed under the name "Victory 76", stated to contain calcium arsenate together with sulfur, nicotine, organic compounds with carbon contents from  $C_{10}$  to  $C_{18}$ , and inert material. Decomposition by nitric and sulfuric acids (4) was shown to be a suitable preparation for the determination of arsenic in this insecticide. An alternative decomposition by bromine was found to be more rapid and to lead to acceptable results, but is judged to be less satisfactory because the decomposition liquid contained suspended dark-colored material, presumably organic bromination products, the presence of which interfered visually at the time the arsenic was reduced and precipitated.

#### PROCEDURES

**SUBSTANCES SOLUBLE IN HYDROCHLORIC ACID.** Dissolve a weighed sample (0.5 to 2 grams) of dried material in a minimal volume of 6 *N* to 12 *N* hydrochloric acid, transfer the solution to a 500 ml. volumetric flask, and dilute to the mark. Transfer an aliquot portion containing about 15 mg. of arsenic to the flask of an all-glass decomposition apparatus with reflux tube, such as that described for use in the determination of arsenic or mercury in organic compounds (3, 4). If a sufficiently sensitive balance is available—e.g., a semimicrobalance—weigh out the whole sample, of such size as to contain about 15 mg. of arsenic, and dissolve in hydrochloric acid. To the solution in the flask add and dissolve rapidly 3 grams of sodium hypophosphite ( $NaH_2PO_2 \cdot H_2O$ ), and then add concentrated hydrochloric acid sufficient to increase the acid concentration to about 6 *N*. Attach the condenser and heat the flask with a small flame, completing the analysis as described (4).

**ARSENICAL MIXTURES CONTAINING ORGANIC MATTER.** *Decomposition by Nitric and Sulfuric Acids (recommended procedure).* Weigh accurately a sample of suitable size (to contain about 15 mg. of arsenic; 0.5 gram of Victory 76) and transfer to the decomposition flask. Add 25 ml. of concentrated nitric acid and warm the mixture for several minutes. Add 20 ml. of concentrated sulfuric acid, evaporate the mixture to fumes, then add more nitric acid and again evaporate to fumes. Allow the liquid to cool partially and introduce 1 gram of ammonium sulfate. When evolution of gas ceases, heat the liquid gently for 5 minutes. Cool, add about 50 ml. of water, and heat until the solution clears or is slightly opalescent. Add 35 ml. of concentrated hydro-

chloric acid, then 3 grams of sodium hypophosphite, and continue as described (4).

*Decomposition of Victory 76 by Bromine.* Transfer weighed sample to the decomposition flask, add 2 ml. of liquid bromine and swirl the mixture for about 5 minutes. Add 50 ml. of hydrochloric acid and heat moderately until nearly all the bromine is expelled (hood). To the cooled solution add 10 ml. of concentrated hydrochloric acid and 3 grams of sodium hypophosphite, and complete the analysis as indicated above.

#### RESULTS

Analytical results for the four materials mentioned are presented in Table I, which includes also comparative results obtained by the familiar distillation procedure (1) selected as the umpire method.

**DISCUSSION.** Results by the reduction method show satisfactory levels of precision and accuracy, and are substantially identical with results by the distillation method. The reduction procedure is the more rapid, requiring about 40 minutes (exclusive of any needed preliminary decomposition), as compared with the 2 to 3 hours required for the distillation procedure.

Table I. Determination of Arsenic in Some Insecticides

Material	Arsenic Found	
	Reduction method %	Distillation method %
Paris green	42.81	42.75
	42.77	42.72
	Av. 42.79	42.57
		Av. 42.68
Lead arsenate	20.25	20.23
	20.18	20.27
	20.15	20.30
	Av. 20.19	Av. 20.27
Calcium arsenate	26.65	26.84
	26.72	26.77
	26.73	Av. 26.81
	Av. 26.70	
Victory 76 insecticide	$HNO_3-H_2SO_4$ decomposition	Bromine decomposition
	3.57	3.64
	3.50	3.55
	3.60	3.60
	Av. 3.56	3.59
		Av. 3.60

<sup>a</sup> Preliminary decomposition by  $HNO_3-H_2SO_4$ .

#### ACKNOWLEDGMENT

Grateful acknowledgment is made to J. J. McGlynn, who executed a series of confirmatory analyses by the reduction and distillation methods.

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# NOTES ON ANALYTICAL PROCEDURES

## A Vacuum Stopcock Lubricant Unaffected by Hydrocarbons

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ESPISTE the frequent need in vacuum work for a stopcock lubricant that is unaffected by hydrocarbons, an inexpensive, readily prepared lubricant does not seem to be described in literature. The glycol citrate polymer of Sager (1) is satisfactory towards hydrocarbons, but its viscosity characteristics make it unsuitable for vacuum work. After a few hours the lubricant flows out of the ground surfaces, and rapid leakage results. In analogy with the common rubber-paraffin lubricant, it appeared that a high-molecular-weight filler might add sufficient viscosity to overcome this difficulty. The addition of cellulose acetate to the glycol ester produced a lubricant that is quite unaffected by hydrocarbons and that permitted the maintenance of a vacuum of  $10^{-4}$  mm. of mercury after more than 6 months' use.

A solution of cellulose acetate was prepared by heating 7.5 grams of Celanese, cut into small pieces, in 45 grams of tetra-

ethylene glycol. After 4 hours at  $140^{\circ}\text{C}$ ., with frequent stirring, the solution appeared homogeneous. Citric acid (30 grams) was heated on an oil bath to  $190^{\circ}$  and the cellulose acetate solution added. Heating was continued at  $180$ – $190^{\circ}\text{C}$ . for 90 minutes.

In order to remove dissolved water, the solution was immediately poured into a previously heated glass jar in a desiccator and the desiccator evacuated as rapidly as foaming permitted. The dehydration has little effect on the final consistency.

Stopcocks lubricated with this material showed no signs of failure after 6 months in contact with liquid toluene, repeated evacuation, and frequent turning. No changes in the properties of the lubricant were observed after storage in a closed jar for a year.

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## Stopcock Lubricants for Use with Organic Vapors

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RECIPIES for lubricants which are insoluble in most organic solvents have been suggested (1–4). Generally, glycerol has been used as a base while bentonite, dextrin, or dextrin mannitol serve as thickening agents. While these undoubtedly behave well under ordinary conditions, sometimes the use of such a lubricant is required in a low-pressure system, where conditions are more exacting. Both bentonite and dextrin are difficult to dehydrate and dextrin is very apt to decompose when heated to the temperature necessary to disperse thoroughly in glycerol. This is undesirable, since water is one of the decomposition products, and while the vapor pressure of anhydrous glycerol is about  $4 \times 10^{-4}$  mm. of mercury at  $25^{\circ}\text{C}$ ., it rises to 0.4 mm. when it contains 2% water. This means that in order to obtain low pressures, long pumping times are necessary.

In an attempt to obviate this difficulty several other thickening agents were tried. The most successful were combinations of mannitol or crystalline carbohydrates such as sucrose with polyvinyl alcohol of medium viscosity. The polyvinyl alcohol made a good thickening agent, but the extra material was required to give the necessary film strength for lubrication.

Before making up, all materials were dried in vacuo at  $70^{\circ}\text{C}$ . for 30 hours. This treatment concentrated glycerol from 94% to better than 99% in about 4 hours and a McLeod gage on the vacuum showed a pressure of  $10^{-4}$  mm. Perhaps the most successful lubricant contained 1 to 3% of medium viscosity polyvinyl alcohol and 15 to 20% of mannitol, in glycerol. After the ingredients had been pasted in the cold, the mixture was carefully heated to about  $130^{\circ}\text{C}$ . and held

there with continuous stirring until the dispersion was uniform and complete. Stirring, when crystals first appeared after the mix cooled, was beneficial in keeping the mannitol finely divided. Although the product was rather dry in appearance, it behaved well after repeated turning of the stopcock.

The mannitol may be replaced with about 40% of sucrose. This preparation behaves well without the polyvinyl alcohol. Sucrose crystals will usually appear in the supersaturated solution after about two days' standing, and stirring for a short time will keep them in a fine state of division.

With either of these lubricants no difficulty was experienced in obtaining and holding a McLeod gage pressure of  $10^{-5}$  mm. They have been used successfully in systems containing ethyl ether vapors. Stopcocks did not have to be regreased more frequently than when normal hydrocarbon greases are used with inert vapors.

Another possible base for this type of lubricant, which could be used in the presence of paraffin hydrocarbons, is triethanolamine. This material has a lower vapor pressure than glycerol ( $7 \times 10^{-5}$  mm. at  $25^{\circ}\text{C}$ .) and the absorption of paraffin hydrocarbon vapors is about the same. In general, the same thickening agents can be used, but more care must be exercised to get the crystals in a sufficiently fine state of division.

Table I shows the comparative absorption of some organic vapors by anhydrous glycerol and triethanolamine, when a 2-gram sample with a surface area of 4.8 sq. cm. was exposed 26 hours at room temperature in a sealed jar containing the liquid organic solvent. The finished lubricants might be expected to absorb considerably less, since they are already saturated with



Table I. Absorption of Organic Vapors

Solvent	Glycerol	Triethanolamine
	Mg.	Mg.
Ethyl alcohol	300	330
Acetone	205	540
Ethyl ether	60	136
Carbon tetrachloride	17	55
Benzene	15	80
Petroleum ether (30-60)	5	5
	Glycerol Base	Apiezon M
Ethyl alcohol	200	2
Acetone	125	20
Ethyl ether	40	160
Carbon tetrachloride	9	940
Benzene	9	410
Petroleum ether (30-60)	2	300

the thickening agent and their viscosity is considerably increased.

The results of a comparison of the glycerol-base lubricant with a standard commercial stopcock grease, Apiezon M, are also shown in Table I. The vapors were absorbed here by a 1-gram sample; otherwise the experimental conditions were identical

with those already described. The solvents used in many of these experiments were chosen to cover a wide range of polarity and no attempt was made to collect compounds to which glycerol was resistant. The lubricant is useless with lower alcohols and ketones where excellent protection is afforded by the Apiezon M. It would probably be of value, however, with the higher members of these families where the solubilities in hydrocarbons become large.

The tests described were severe, since the area exposed to vapor is many times that obtained with an ordinary stopcock ground-glass joint. The effect of fifty liquid solvents on this type of lubricant has been recorded (4). The absorption results here are in good agreement.

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## Determination of Phthalate

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**N**UMEROUS ternary systems involving phthalates have been investigated in this laboratory during the past 20 years. The analytical procedure for the determination of phthalate has necessarily been an indirect one, since no direct method known to the authors is satisfactory in aqueous solution.

Kappelmeier (3) determined phthalate in alkyd resins by the precipitation of potassium phthalate containing one molecule of alcohol of crystallization. This precipitation is carried out in benzene or in anhydrous alcohol-ether solution and is not applicable to aqueous solutions. Fonrobert and Muenchmeyer (2) determined phthalate in varnish and Thames (4) in plasticizers by precipitation of lead phthalate with lead acetate and conversion of this precipitate into lead sulfate which was weighed.

Zombory (5) found that lead could be determined gravimetrically by precipitation as lead phthalate in alcoholic solution. It was felt that possibly this procedure could be reversed and phthalate be determined by adding lead as the precipitating reagent. With this thought in mind, this investigation was undertaken.

Solubilities of lead phthalate in water and in various concentrations of alcohol were determined at various temperatures. It was found that at 25° C. the solubility of lead phthalate in 33% alcohol by volume is 2 mg. per 100 cc. of solution. The solubility does not decrease appreciably in higher concentrations of alcohol.

Both lead nitrate and lead acetate were independently used as precipitating agents, the former giving low results and the latter high results.

It is apparent that when lead nitrate is used as the precipitating agent, nitric acid is one of the by-products. The resulting solution is therefore acidic, accounting for the increased solubility of the lead phthalate which is a salt of a weak acid and consequently soluble in a strong acid.

An investigation was therefore made to find the optimum pH for precipitation. A solution made up of lead nitrate and excess sodium phthalate in 33% alcohol by volume as used by Zombory (5), showed a pH of 7.6 using the glass electrode. Determinations were then made using sodium phthalate and a calculated excess of lead nitrate in alcoholic solution of the same strength but with varying acid concentrations. From pH 2.8 to 6.4 low results were obtained. Above 6.4 high results were obtained.

This is explained by the solubility of lead phthalate in acid solution and the precipitation of lead hydroxide as the alkalinity increases. This latter fact was substantiated by determining that lead hydroxide starts to precipitate from alcoholic solutions when the pH reaches 5.1. Britton (1) found that lead hydroxide comes out of aqueous solution at a pH of about 6.

It is evident that the optimum pH value at which phthalate should be determined overlaps the pH value at which lead hydroxide forms. One cannot hope to make these two errors self-compensating, since excess lead nitrate will be present in varying amounts in determinations of phthalates in unknown solutions.

It seemed that by using lead acetate as the precipitating agent in place of lead nitrate, the by-product of the reaction would be the weak acetic acid and better results might be obtained. A new difficulty was encountered here, since the precipitate came down very finely divided and did not settle out upon standing, rendering filtration and washing virtually impossible.

A few determinations were completed by making the precipitation in aqueous solution and boiling the mixture before the addition of alcohol. After standing, the mixture was centrifuged and the precipitate washed and weighed. All the values obtained were high and the magnitude of the errors was not consistent. This is believed to be due to the contamination of the precipitate with varying amounts of basic lead acetate.

The evidence indicates that phthalate cannot be determined directly by the addition of lead nitrate or acetate to aqueous solutions containing phthalate ion.

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THE material for this paper was taken from a thesis of John F. Stremper presented to the Graduate Committee of Trinity College in partial fulfillment of the requirements for the master of science degree.



# Stability of Wijs Solution for Iodine Number Determinations

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THE Wijs solution is probably the most satisfactory for general use in determining iodine numbers. Its more widespread utilization is hindered mainly by its supposed difficulty of preparation and short life. The first objection is hardly valid if chlorine is available and if the analyst is reasonably careful. The short life would appear to be a much more serious objection, since three standard references on fat analytical methods specifically caution against using this solution when it is more than 30 days old (1, 2, 4). However, Hilditch (3) does not accept this view, and Wijs himself (5) claims indefinite stability of the reagent. Previous experience by one of the present authors is in agreement with the two last-named investigators. Since a decision on the stability of Wijs solution was considered necessary in connection with some analytical work, the authors measured the stability of the reagent when stored at room temperature in 250-ml. dark bottles. The solution was prepared in the standard manner and contained 1.5% excess iodine equivalents of iodine over chlorine. Linseed oil, stored in the box, was used as the test substrate.

Over a total period of 505 days, the Wijs solution did not change sufficiently to cause a measurable difference in the iodine number of the substrate. No measurable differences were found when the reagent was taken from bottles that had been previously opened. These facts indicate the validity of storing the solution a year or more, if storage is in small bottles which are opened as needed.

Table I. Stability of Wijs Solution

Bottle No.	Age of Solution, Days	Days Since Bottle First Opened	Iodine No.
1	6	0	177.3
2	45	0	178.0
2	97	52	177.1
3	97	0	176.5
3	132	35	176.8
3	174	77	176.3
3	231	134	177.0
4	277	0	177.4
4	314	37	177.0
4	374	97	177.8
5	404	0	176.8
5	455	51	177.2
5	505	101	177.2

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PAPER 52, Journal Series, Chemical Research Department, General Mills, Inc.

## An Aid in Ashing Certain Materials

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THE ashing of many products is often difficult and time-consuming, as is evidenced by the number of procedures published for special materials (1). This is especially true for substances that are finely ground, contain oil or fat, or have a high phosphorus-to-base ratio. For such materials, a simple procedure which was recently devised to ash large volumes of dried egg white (2) is suggested.

This procedure consists of lining the crucible with filter paper, placing the material over a Meker burner in this lined crucible, moistening the char with a solution of magnesium chloride, and completing the ashing in a muffle furnace.

Filter paper of the correct size (Whatman No. 40 or equivalent) is folded as for filtering, the tip of the cone folded back, and this lined cone fitted into the crucible. This liner prevents overheating because of the air space between it and the crucible and permits the easy escape of the volatile materials,

since the charring proceeds slowly from the outside toward the center. The charred mass tends to retain its cone shape and is free of the crucible. Thus, the crucible walls are protected from the action of the materials ashed. Fatty materials burn more evenly and slowly, without spattering. The char is moistened with a solution of magnesium chloride added dropwise over the entire mass and ashed in a muffle overnight at 600° C. The ash obtained is light, fluffy, and voluminous and dissolves readily in dilute hydrochloric acid on heating.

If unaided by this procedure, materials which have a high phosphorus-to-base ratio fuse on ignition to a glassy mass which frequently entraps carbon that can then be burned off only with great difficulty. This fusion is caused by the conversion of the dihydrogen and monohydrogen phosphates upon ignition to the metaphosphate (3), which does not dissolve readily in hot dilute hydrochloric acid. In the suggested procedure, magnesium chloride supplies base to produce the tertiary phosphate which is unaffected on heating to 600° C.

In the authors' experience, samples ash poorly if they do not contain ample base for the phosphorus or in the case of plant material if the stalks, stems, hulls, coatings, or bran have been removed. In these materials, the use of the lined crucible and magnesium chloride is beneficial. In comparative trials, the proposed method aided the ashing of fresh tissues such as muscle, liver, intestines, and fat of chicken, pork, and beef as well as fresh egg white and yolk, casein, lecithin, corn gluten meal, flour, and starch.

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JOURNAL Article No. 697 (n.s.) Michigan Agricultural Experiment Station.







## A Constant-Rate Dropping Funnel

CHEMISTS are often annoyed to find, after carefully adjusting a dropping funnel to deliver a liquid dropwise into a reaction mixture, that within a short time the flow of drops has either stopped entirely or greatly diminished. A combination of two factors may be held largely responsible for this: the diminishing hydrostatic pressure as the liquid level falls, and the gradual tendency for the stopcock to close during the flow of the liquid. This conclusion can be readily verified by experiment.

A simple modification eliminates both these factors from an ordinary dropping funnel.

The modification, as shown in the accompanying diagram, chiefly consists in placing tightly in the top of the funnel a one-hole stopper, *A*, in which is inserted a glass tube, *B*, reaching nearly to the bottom of the

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funnel. When liquid is allowed to flow out, air enters tube *B* and escapes at *D*. The pressure in the air space, *C*, then changes in such a way that as the liquid level drops, the sum of *C* and hydrostatic pressure represented by the distance *DE* remains essentially constant. This is in accordance with the principle of the Mariotte flask (*1*).

The gradual tendency of the stopcock to close may be obviated by restricting the entrance of air into *B* and then opening the stopcock *G* completely. The air flow can be controlled either by means of a capillary tube attached to *B* or, as illustrated, *H*, by a screw clamp on a short piece of rubber tubing into which has been inserted a small wire, *I*. If the opening at *F* is of large diameter, it is sometimes necessary to draw out the tube in order to prevent air from entering at this point.

Dropping funnels equipped in this way have been found useful in this laboratory for the dropwise delivery of liquids in reaction mixtures. Although a slight decrease in rate can be noticed during the time of delivery, the flow is constant enough for most purposes.

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## NEW EQUIPMENT

### Sulfur Train

Numbered among improved apparatus accelerated by the war program is a sulfur train, originated by the Universal Oil Products Co. and built by the Precision Scientific Co., 1736 North Springfield Ave., Chicago. The method, identified as U.O.P. Method H-201-43-A, determines 0.0002 to 0.05% of sulfur in combustible liquids and gases. The samples are burned in a stream of purified air and the products of combustion absorbed in a solution of sodium hypobromite. The sulfur is then determined as sulfate by turbidimetric measurement of colloidal barium sulfate.

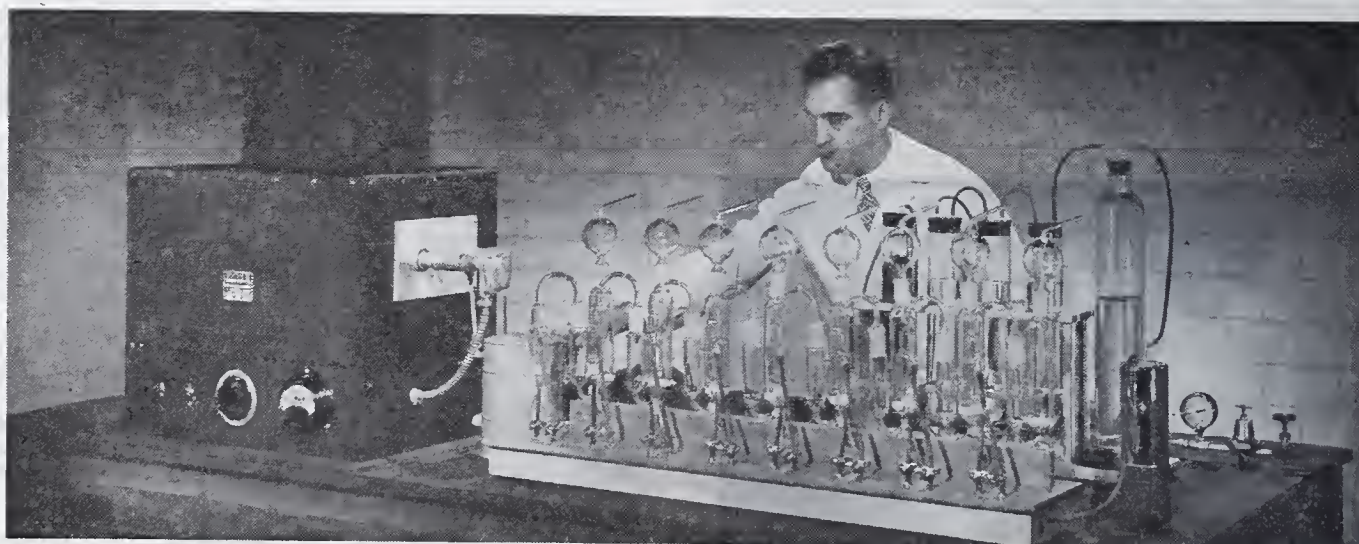
The apparatus consists of an air-pressure regulator and filter pressure gage, air-purification furnace, cooling tank, gas scrubbers, water regulator, moisture trap, and bank of eight A.S.T.M. sulfur lamps.

Compressed air controlled by a pressure-reducing valve is reduced

to 2 p.s.i., then passes through an activated charcoal tower and into the air-purification furnace containing a heated stainless steel U-tube 1 inch in diameter and approximately 4 feet long, filled with quartz chips.

Air leaving the furnace is cooled to approximately room temperature by passing through a water-jacketed copper-coil condenser at the furnace outlet. It then passes successively through water scrubbers, sodium hypobromite, and sodium hydroxide scrubbers, then through a constant-pressure regulator, into a moisture trap fitted into a vacuum bottle, and finally into a distribution manifold serving the sulfur lamps.

For the determination of sulfur in gases, the apparatus includes eight Duralumin weighing bombs of 35-ml. capacity, for connection to the sulfur lamps.





## Sampling and Analysis of Anhydrous Hydrogen Fluoride

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THE production and use of anhydrous hydrogen fluoride in tank car quantities require the development of an accurate procedure for its sampling and analysis.

On account of the volatility of anhydrous hydrogen fluoride (boiling point  $19.4^{\circ}\text{C}.$ ), the serious danger of burns, and the tendency of impurities to segregate, a correct and safe sampling procedure is absolutely essential. Strict adherence to the procedure described here yields excellent results.

## SAMPLING

To obtain a proportional sample from the pipeline leading from the storage tank to the tank car into an evacuated, or vented, H-type cylinder fitted with valve and coupling suitable for anhydrous hydrogen fluoride. To accomplish this the cylinder must be weighed on a scale and weighed, all connections made gastight, the valves opened slightly to bleed off 1 pound for every 1000

An accurate and safe procedure for the sampling and analysis of anhydrous hydrogen fluoride from tank cars and cylinders is described.

pounds of the hydrogen fluoride run into the tank car. *Caution!* A cylinder must never be filled to more than 85% of its water capacity.

**APPARATUS.** From H-cylinder samples draw a smaller sample from the liquid phase only into an evacuated or cooled E-type cylinder and fill to 85% of its water capacity. This is done by tilting the H-cylinder sufficiently to ensure the drawing of liquid hydrogen fluoride only, connecting to an evacuated E-cylinder, and allowing about 3 kg. (6 pounds) to flow in. This is the sample taken to the laboratory. Only the liquid phase is sampled, since it constitutes about 99.96% of the net weight of the shipment.

Draw a sample for analysis from the 6-pound sample cylinder; in order to do this fairly the following special equipment is necessary:

1. A rubber weighing tube (Figure 1) is machined from a 5-cm. (2-inch) (iron pipe size) extra-heavy-wall hard-rubber pipe. A narrow ledge on the inside is machined 12 cm. (4.875 inches) above the bottom. The lower end is plugged with a hard-rubber disk and the entire inside is coated with a heavy layer of high melting point wax (Quaker State M wax 165 dark amber). The top end is stoppered with a removable No. 10 $\frac{1}{2}$  solid rubber stopper which has been wax-treated. The removable metal part consists of a metal tube with coupling at the upper end, a metal hook silver-soldered to the tube just below the coupling, a perforated metal grid which slides on the tube, and a grid stop, silver-soldered at the end to prevent it from sliding off.

The whole metal assembly of the sampling device must be gold-plated or, better, made of platinum. On account of contact with ice and hydrofluoric acid, the metal assembly cannot be of cadmium, brass, copper, iron, or steel, since corrosion of these metals contaminates the sample, with the possibility of giving high fluosilicate and sulfuric acid results. Monel metal is passable but preferably it should be gold-plated. The metal assembly should not be left in contact with the acid any longer than necessary.

2. A torsion balance with a sensitivity of 15 mg. and a capacity of 500 grams.

3. A sturdy support for the E-type cylinder to hold it horizontally, as shown in Figure 2.

Before drawing a sample for analysis, set up the sample cylinder on the support in a sufficiently inclined position to ensure withdrawal of the liquid phase only. Cylinders filled to 85% of water capacity can be placed in a horizontal position, but if many samples are to be taken the cylinder should be tilted slightly. Below place a Harvard trip balance (sensitivity need be no greater than  $\approx 1.0$  gram), so that the left pan is in line with the cylinder valve outlet. Set the rubber weighing tube on the left pan and add enough weights to the other pan to overbalance, in order to hold the weighing tube at its highest position on the balance. Loosely couple the metal tube assembly to the cylinder adapter, then raise or lower the balance, so that the hook on the metal tube is in line with the top of the weighing tube. *Caution!* This adjustment is necessary to prevent suckback of liquid into cylinder valve. Make certain the cylinder clamps are secure in order to prevent slipping when the cylinder valve is opened. The entire setup must be under a good hood.

**SAMPLING PROCEDURE.** The metal part and rubber stopper may be dried in the oven, but the weighing tube must be dried at room temperature by forcing dry air into it until globules of water or moist areas are no longer visible. Rubber weighing tubes after repeated use should be rewaxed when there are any bare spots in the coating or when any odor of hydrogen fluoride is detected in the dry tube.

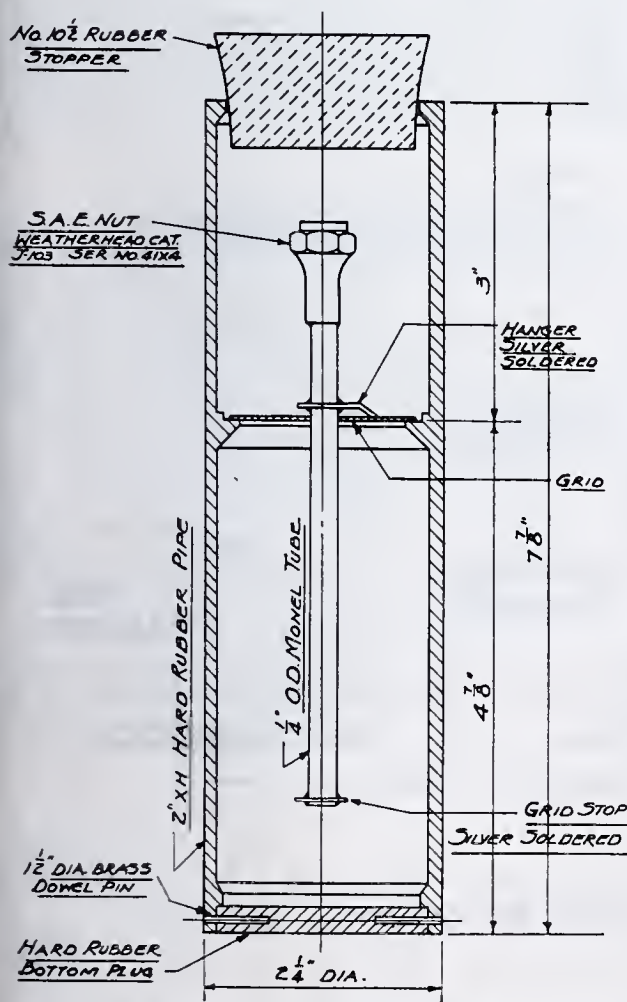


Figure 1. Assembly of Acid Container

Metal part to be gold-plated inside and out



Weigh the entire dried weighing tube (metal tube and rubber stopper included but not assembled) on the torsion balance, using rough weights, or make up an approximate tare weight and balance exactly with the sliding weight. Test the accuracy of this torsion balance for such factors as equality of arms, positioning of weights, etc., and make suitable corrections if the errors are in excess of 20 mg. After thus establishing the weight of the entire weighing tube, do not disturb the tare weights, and from this point on make all succeeding weighings by adding analytical weights. Complete the following operations as quickly as possible:

Place 80 grams of chopped ice in the bottom part of the weighing tube, and properly insert the metal tube, so that the perforated grid rests flat on the narrow ledge and the hanger rests over the rim of the rubber tube. Add 50 grams more of chopped ice to the top part of the tube above the grid. Wipe off any droplets of water on the outside of the weighing tube or on the coupling. Weigh the entire assembly exactly including rubber stopper, adding only analytical weights, and record the total weight of ice added.

The 80 grams of ice in the bottom serve to absorb the heat of dilution of the hydrogen fluoride and the 50 grams in the top serve to trap any vapors formed through local concentration of heat. The entire weight of ice must not greatly exceed 130 grams, as this amount when melted plus about 40 grams of sample will not bring the liquid level above the outlet of the metal tube. If the setup is exactly as described above, a clearance of about 5 cm. (2 inches) is assured. *Caution!* Never allow the metal tube outlet to be submerged during addition of the sample, for a very rapid suckback which surely will follow will ruin the sample and may cause an explosion.

Cautiously open the valve (always wear rubber gloves) of the sample cylinder already set up and allow a few milliliters of the hydrogen fluoride to flow out into a Monel waste beaker to sweep the outlet free from condensed water that may have lodged there in. Place the weighing tube (without the rubber stopper) on the Harvard trip balance and immediately couple the metal tube to

the cylinder adapter, tightening with a wrench (Figure Balance with the rough weights, make certain that the metal tube does not hinder the balance swing, then add 40 grams more. Carefully open the cylinder valve slightly and adjust the flow of hydrogen fluoride to about 10 grams per minute. As the hydrogen fluoride strikes the ice a sizzling sound may be fairly heard and through this guidance the rate of flow may be varied. During the flow, watch the top of the weighing tube for escaping vapors and as soon as any are seen cut down the flow. Keep testing the balance swing, to make certain shifting ice in the upper part does not cause sufficient friction against the metal tube to hinder the swing. Keep running in the sample until 40 grams are approximately balanced, close the valve, and wait 15 seconds for drainage. Disconnect the metal tube and discharge carefully into the weighing tube, then stopper tightly with delay.

Weigh exactly on the torsion balance, adding only analytical weights, and record the additional weight over the ice weight as the sample weight. Mix thoroughly by careful inversion until all the ice melts, being certain to keep the tube tightly stoppered, so that none of the solution is lost before the ice melts and the solution becomes homogeneous. Remove the metal tube and restopper without delay to prevent escape of sulfur dioxide. The well-mixed diluted acid clinging to the metal tube will be of no consequence, since aliquot weights will be taken for analysis. Clean and dry the metal tube at once. Proceed with the analysis as directed below without delay.

#### ANALYSIS

**SULFUR DIOXIDE.** This must be the first constituent determined, because opening the weighing tube for taking the other aliquot samples may result in loss of sulfur dioxide.

Provide a well-waxed 250-ml. beaker and a Bakelite stirring rod. To this beaker add 50 ml. of water and exactly 10 ml. standard 0.1 N iodide-iodate solution (3). Weigh on a torsion balance. Place a 50-gram weight on the balance pan, then po

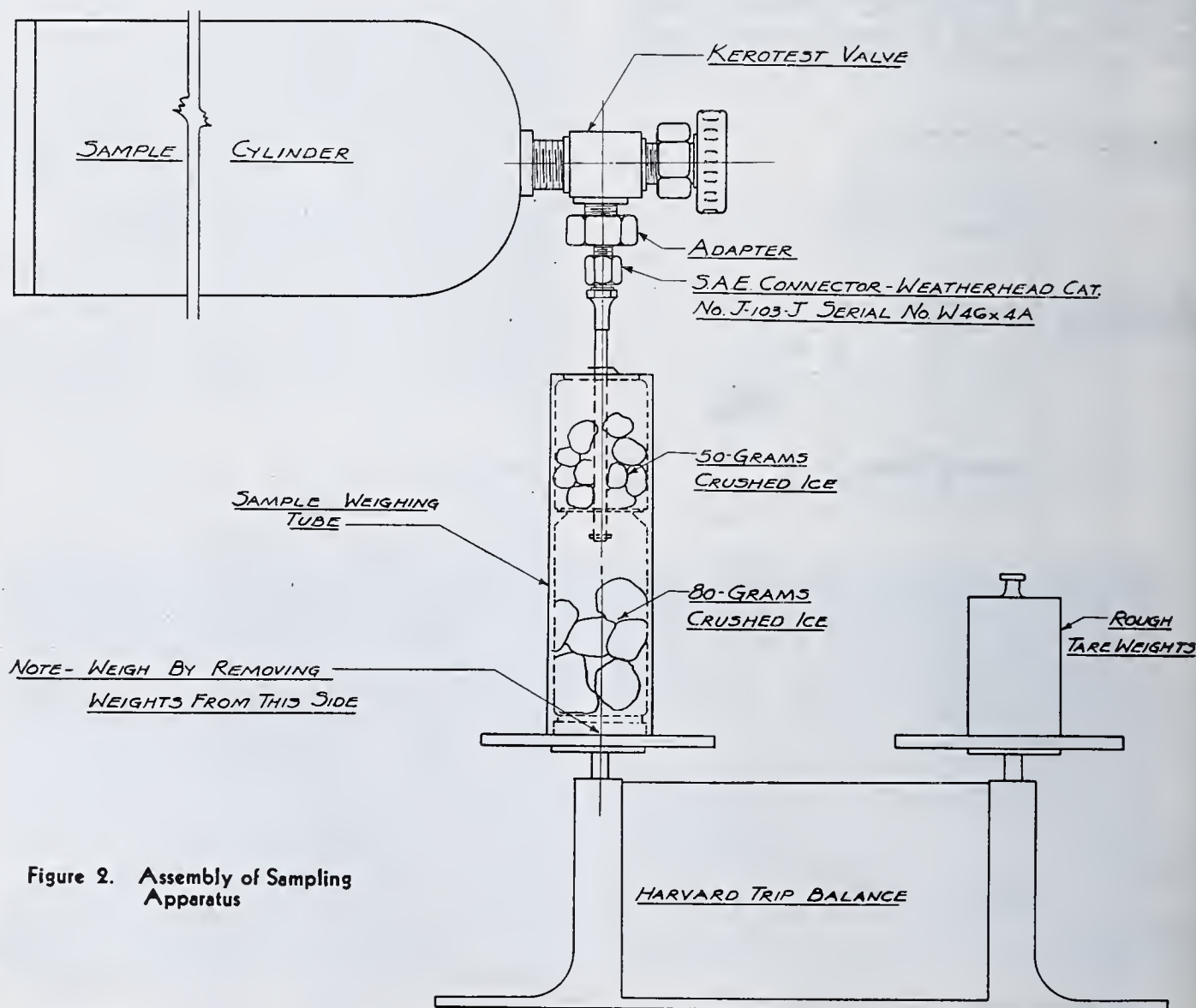


Figure 2. Assembly of Sampling Apparatus



aliquot portion of the sample into beaker carefully until slightly over-  
nced. Weigh accurately to  $\pm 0.5$   
n. Back-titrate the excess liberated  
e with standard 0.1 N thiosul-  
t using starch solution as indicator.  
o color appears upon adding starch,  
te with iodide-iodate to a blue

andardize the iodide-iodate solu-  
against the thiosulfate under like  
itions, substituting 50 ml. of water  
the sample and making slightly  
with pure hydrofluoric acid. Cal-  
te to sulfur dioxide and report to  
significant figures.

l. of N thiosulfate =  
0.032 gram of  $\text{SO}_2$

alculation to per cent sulfur dioxide  
ased on the following equation  
h also applies to succeeding calcu-  
ns:

$$\frac{\text{grams of ice} + \text{grams of anhydrous HF)} \times (\text{factor } X) \times}{(\text{ml. of standard solution}) \times 100} = \% X$$

weight of aliquot)  $\times$  (grams of anhydrous HF)  
or  $X$  = normality of standard solution  $\times$  normal equivalent

TOTAL ACIDITY. Weigh a 12-ml. platinum weighing bottle  
ure 3) on the analytical balance. Add as quickly as possible  
o 45 drops of the sample solution by means of a Bakelite  
ping pipet, cover the weighing bottle promptly, and re-  
h. In a 250-ml. waxed beaker place 100 ml. of water and 1  
of phenolphthalein indicator and make faintly pink with  
V alkali. In this submerge the weighing bottle, knock off  
cover, and titrate with standard 0.5 N alkali to a permanent  
color. Approach the end point slowly by adding a fraction  
drop of alkali at a time. If the pink color fades, wash the  
ion into a plain beaker, heat to about 60° C., and continue  
titration to a permanent pink. A fading end point is due to  
ilicate, which may be in the acid or formed during the titra-  
from silica in the standard alkali. Silica in the standard  
i does not affect a total acidity determination if the heating  
ution is taken near the end point, but does cause erroneous  
es in determination of fluosilicic acid.

alculate the total acidity to hydrogen fluoride and report to  
e significant figures. Corrections for the impurities will be  
e later.

1 ml. of N alkali = 0.01999 gram of HF

LFURIC ACID. Weigh on the torsion balance a 50-gram ali-  
of the sample into a 75-ml. platinum dish and evaporate to  
rent dryness on the water bath. Add 10 ml. of water,  
orate again to dryness on the water bath, and note as evap-  
on progresses whether any odor of hydrofluoric acid can  
etected. Repeat as often as hydrofluoric acid could be  
et in the previous evaporation. Usually two evaporations  
water are sufficient when the sulfuric acid content is below  
. When all the hydrofluoric has been expelled, add 25 ml.  
water and titrate with 0.1 N alkali, using phenolphthalein as  
ator. The titration is equivalent to sulfuric acid and fluo-  
nic acid. The latter decomposes to sulfuric acid upon  
oration with water.

alculate to sulfuric acid and report to two significant figures  
uric acid plus fluosulfonic acid) as sulfuric acid,

1 ml. of N alkali = 0.049 gram of  $\text{H}_2\text{SO}_4$

the presence of aluminum or iron is suspected, add 1 gram of  
al c.p. sodium fluoride fluosilicate-free, before titration.

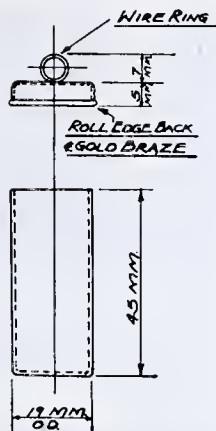


Figure 3. Platinum Weighing Bottle  
Approximate weight, 22 grams

If heavy metals, such as copper, lead, nickel, etc., are present, add 1 gram of neutral potassium oxalate before titration.

FLUOSILICIC ACID. Weigh on a torsion balance a 50-gram aliquot sample into a 75-ml. platinum dish. Add 0.2 gram of potassium chloride, stir with a platinum rod until the salt dissolves, and evaporate to dryness on the water bath. Dissolve the residue in 25 ml. of water. Add 2 grams of potassium chloride, and cool to below 10° C. (4). Add 1 ml. of phenolphthalein indicator and titrate with silica- and carbonate-free 0.5 N alkali. This titration should preferably be carried to just short of the end point and finished with silica- and carbonate-free 0.1 N alkali. During the entire titration do not allow the temperature to rise above 10° C.; keep the dish surrounded with chopped ice. Disregard the amount of alkali required, for the acidity is due to acid fluoride with which we have no concern except to neutralize it. Heat the dish to at least 60° C. and titrate the hot solution with 0.1 N alkali to the first faint permanent pink color.

If means of cooling for the first titration is not conveniently available, add 35 ml. of ethyl or methyl alcohol, stir somewhat, let stand 15 minutes for potassium fluosilicate to precipitate, and titrate at room temperature as above. After reaching the end point wash the solution into a beaker, boil 10 minutes, and titrate the hot solution with 0.1 N alkali as above.

The hot titration is equivalent to four of the six fluoride atoms in the fluosilicic acid. Multiply the hot titration by 3/2 to obtain the total milliliters of alkali for fluosilicic acid. Calculate to two significant figures.

1 ml. of N alkali = 0.024 gram of  $\text{H}_2\text{SiF}_6$

The equations involved in fluosilicic acid determinations are as follows:



Fixation of the fluosilicate as potassium fluosilicate permits the use of a large sample and the removal of the bulk of the hydrogen fluoride by evaporation. This procedure avoids a cumulative error due to the traces of silica in the large quantities of alkali which would otherwise be required to neutralize the hydrogen fluoride.

In the procedure potassium chloride is added so that the almost insoluble potassium fluosilicate does not hydrolyze below 10° C. and therefore is not titrated. The addition of alcohol causes quantitative precipitation of potassium fluosilicate and therefore it is not titrated at room temperature. The disadvantage of using this alternative modification is the necessity of boiling off most of the alcohol before titrating the fluosilicate.

While the fluosilicic acid, becomes fixed as potassium fluosilicate, at least a substantial part of the potassium chloride is converted to potassium hydrogen fluoride. It is neutralized in the

Table I. Recovery of Silica Added to Hydrofluoric Acid

48% HF Grams	Added Gram	SiO <sub>2</sub> Found Gram	H <sub>2</sub> SiF <sub>6</sub> in 100% HF	
			Added %	Found %
50	None	<0.0003	None	<0.001
50	0.0369	0.0369	0.37	0.37
50	0.0335	0.0337	0.34	0.34
22	0.0059	0.0058	0.13	0.13
22	0.0044	0.0046	0.10	0.10

Table II. Analyses of Hydrogen Fluoride in Tank Cars

	Car A		Car B		Car C		Car D		Car E		Car F		Car G		Car H	
acidity as	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
ities as HF	99.9	99.9	99.75	99.79	99.8	99.6	100.0	99.9	100.0	100.0	99.70	99.90	99.8	99.9	99.9	99.84
HF	0.11	0.11	0.17	0.16	0.088	0.075	0.049	0.049	0.14	0.11	0.047	0.044	0.042	0.039	0.05	0.06
	99.8	99.8	99.6	99.6	99.7	99.5	99.9	99.8	99.9	99.9	99.6	99.8	99.8	99.9	99.9	99.8
	0.096	0.098	0.17	0.16	0.074	0.069	0.041	0.042	0.13	0.13	0.058	0.059	0.048	0.052	0.056	0.057
O <sub>2</sub> + H <sub>2</sub> SO <sub>4</sub> )																
H <sub>2</sub> SO <sub>4</sub>	0.059	0.060	0.027	0.026	0.025	0.031	0.023	0.015	0.022	0.019	0.006	0.006	0.019	0.006	0.018	0.008
	0.027	0.027	0.021	0.022	0.039	0.023	0.017	0.017	0.056	0.033	0.011	0.006	0.005	0.004	0.013	0.018
st	M	M	M	M	M	M	M	M	M	M	M	M	F	F	S	S



cold titration, but if the alkali used to neutralize it contains silicate, the silica will also become fixed as potassium fluosilicate and high results for the subsequent hot titration ensue. The magnitude of this error depends upon the length of time the standard alkali had been stored in glass and, of course, the amount used in the cold titration. To eliminate this source of error the standard alkali must be stored in a heavily waxed bottle (high melting point mineral wax) or better still, in a steel drum. If stored in a steel drum, it must be siphoned over through an iron or nickel tube. An alkali-filled scrubber should be attached to the drum to remove carbon dioxide from air entering as solution is removed.

This procedure is a modification of the method given by Kolthoff and Furman (2).

The method is capable of giving results of considerable accuracy, as shown by experiments in which known amounts of silica were added to reagent hydrofluoric acid. Optical quartz ground to 100-mesh, washed with hydrochloric acid, and ignited was used. Harshaw reagent hydrofluoric acid was added to

the quartz and allowed to stand at room temperature with frequent stirring until the quartz dissolved before proceeding with the method. Results are shown in Table I.

**HYDROFLUORIC ACID.** To obtain the hydrofluoric acid content deduct from the total acidity the sum of  $0.8330 \times \% \text{H}_2\text{Si}$ ,  $0.4078 \times \% \text{H}_2\text{SO}_4$ , and  $0.6243 \times \% \text{SO}_2$ .

(Note. The factors used throughout this procedure and in standardization of the alkali are based on rational atomic weights, inasmuch as the atomic weights of hydrogen and fluorine are low.)

Table II shows the results of duplicate analyses of eight different tank cars of anhydrous hydrogen fluoride by this method.

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## Infrared Analysis of Butadiene

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IT HAS been pointed out many times in the literature (1-5, 7, 8) that the infrared absorption spectrum of an organic molecule is unique, and that with few exceptions admixture with other compounds does not affect this property. As a result, the concentration of any component in a mixture can be determined by infrared analysis, provided it has at least one absorption band at a wave length where the other components are relatively transparent.

Within experimental error, the absorption measurements made on the usual research type of infrared spectrometer follow the familiar Beer's law,

$$I = I_0 e^{-k\lambda c x}$$

where  $I_0$  is the energy incident on the sample,  $I$  is the energy transmitted by the sample,  $k$  is the absorption coefficient at wave length  $\lambda$ ,  $c$  is the concentration, and  $x$  is the length of the optical path through the sample usually equal to the length of the absorption cell. This expression can be put in the alternate form:

$$\log_{10} \frac{I_0}{I} \equiv \log_{10} \frac{1}{T} \equiv \epsilon C X \equiv D$$

where  $T$  is the transmission fraction,  $\epsilon$  is the extinction coefficient, and  $D$  is the optical density. For monochromatic radiation the optical density of a mixture equals the sum of the optical densities of the components—that is, at any wave length  $\lambda$ :

$$D = d_1 + d_2 + d_3 + \dots + d_n$$

where  $D$  is the optical density of the mixture and  $d_n$  is the optical density of the  $n$ th component. In general,  $n$  such equations, one for each of  $n$  selected spectral positions, are required to determine the concentration of each component present.

It frequently happens in industrial work that the concentration of only one component present in a mixture is of interest. In such cases the analytical procedures can often be simplified. This paper discusses such a problem, the infrared analysis of refined butadiene.

Refined butadiene contains varying amounts of impurities such as butene-1, butene-2, perhaps traces of isobutylene and acetylenes, together with small amounts of saturates, the latter usually amounting to less than 5% of the total impurities present. These impurities not only absorb strongly at 6.9 microns ( $1450 \text{ cm}^{-1}$ ) where butadiene is relatively transparent, as shown in Figure 1, but the optical densities of the major impurities, butene-1 and

butene-2, are of the same order of magnitude (Figure 2). As a consequence, as long as the concentration of the combined impurities remains constant, fluctuations in their relative amounts will have no significant effect on the optical densities of refined butadiene samples. It is obvious, therefore, that the total i

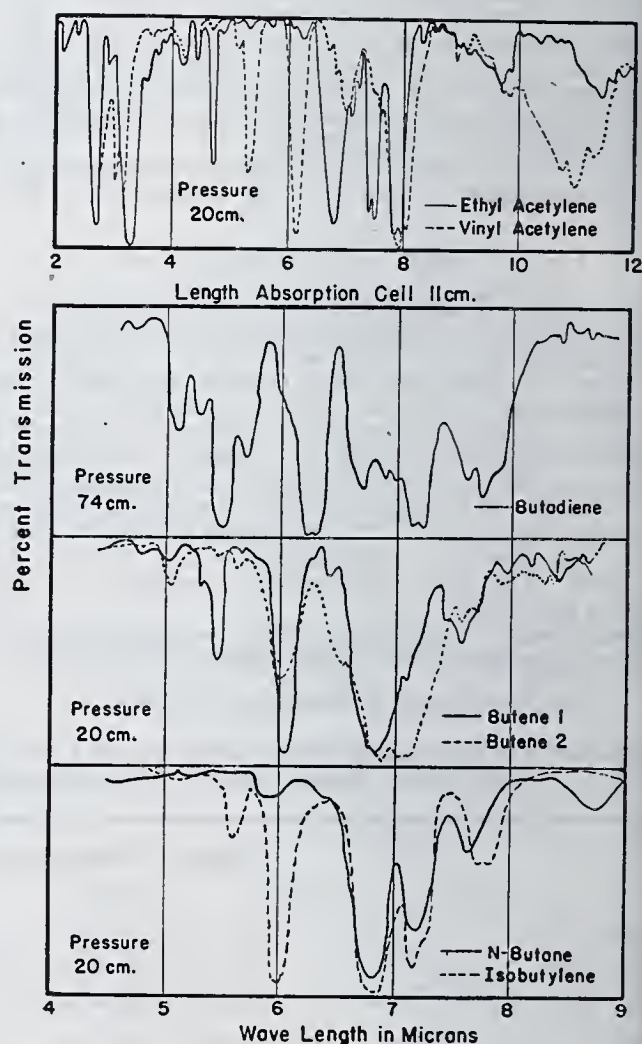


Figure 1. Infrared Absorption Curves of Butadiene and Associated Impurities



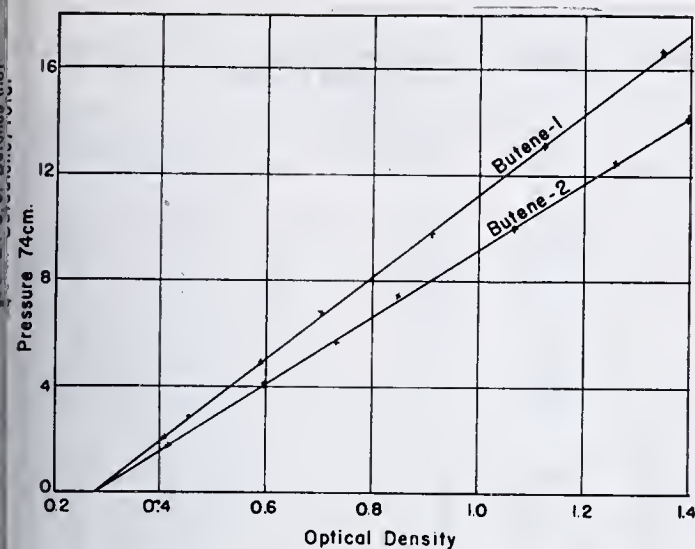


Figure 2. Transmission of Butadiene-Butenes Mixtures at 6.9 Microns

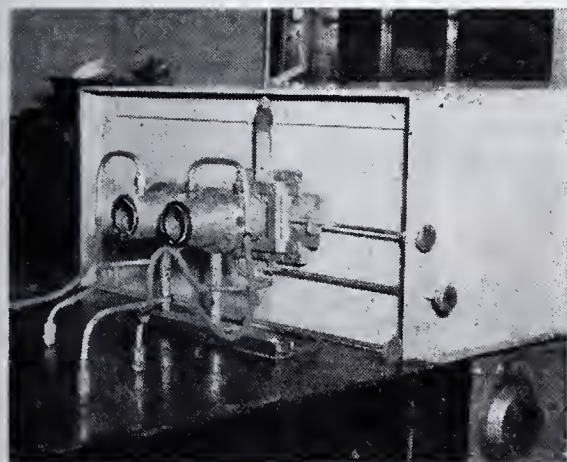


Figure 3. Infrared Absorption Cells and Shutter Mechanism

ies can be determined from the transmission of the sample at microns, the butadiene being determined by difference. This procedure is to be preferred over that of determining the butadiene concentration directly because a consideration of the exponential nature of Beer's law makes it evident that the precision of direct analysis for a component falls off as its concentration approaches 100%.

#### EXPERIMENTAL

Results were obtained with two infrared spectrometers. A Littrow-type, automatic recording spectrometer of 1-meter length which has a  $100 \times 150$  mm. rock salt prism was used to develop the analytical method. This instrument uses a photoelectric potentiometer (6) and a Leeds & Northrup Speedomax to record the transmission curves. The plant control instrument, a  $60 \times 75$  mm. rock salt prism, is not automatic, but was designed to record the transmission of samples at a predetermined wavelength (6.9 microns for butadiene analysis) by means of a photoelectric potentiometer (6) and a Leeds & Northrup Microphotometer. Further constructional details of the plant control instrument are illustrated in Figures 3 and 4. Figure 3 shows the two gas-absorption cells, sample, and dummy, as well as the shutter mechanism. This mechanism, also presented in Figure 4, is arranged so that a 12-mesh stainless steel screen can be inserted between the spectrometer and the absorption cells. The only function of this screen is to decrease the amount of light entering the spectrometer; it is evident that, after standardizing its transmission against butadiene of known purity the screen can then be used as a comparative standard for butadiene analysis. The transmission of the screen is checked frequently in the plant, usually every 4 to 6 hours.

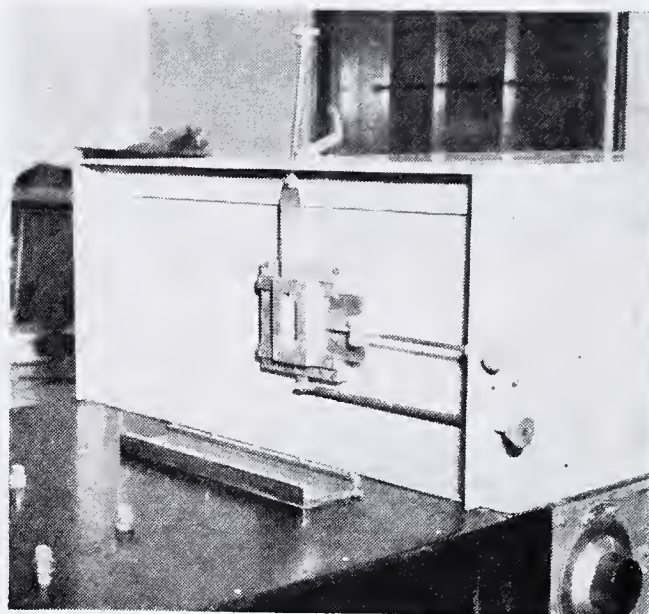


Figure 4. Infrared Shutter Mechanism

For plant analysis the feed-back control on the photoelectric potentiometer is set at a maximum. The source and slit widths are then adjusted until nearly full-scale deflection is obtained with butadiene in the sample cell or with dry air, in either the sample cell or dummy and the absorption screen in place.

After standardizing the absorption cells using the screen and dry air the analysis of butadiene is carried out as follows: Continuously flowing streams of gaseous butadiene are brought to a distribution manifold near the spectrometer. The sample passes from the manifold over solid potassium hydroxide into the sample

absorption cell, thence exits through the barostat shown in Figure 5 to the atmosphere. The barostat acts as an exit valve on the absorption cell and at the same time adjusts the pressure of the butadiene to a constant value. The sample must be introduced with sufficient force to flush out previous samples. This operation is usually completed in less than 2 minutes. At the end of this time the butadiene flowing through the sample cell is turned off. While the flushing operation is being carried on the instrument zero is recorded. Next the shutter is opened and the transmission of the sample is recorded for about 2 minutes. The shutter is then closed, the absorption screen lowered into place, the sample cell moved out of the optical path, and the dummy moved in, the shutter is opened, and the transmission of the dummy and screen is recorded for the next 2 minutes. Duplicate determinations are made. The instrument zero is subtracted from the recorded transmission for each sample and as a convenience, this is reported in terms of meter deflection. The purity of the sample is determined from the work curve shown in Figure 6, but a correction equal to the difference between the "standard equivalent" value of the absorption screen and the "equivalent" value of the screen found following each analysis must be applied when necessary. Duplicate analysis including all calculations can be made in less than 10 minutes.



Figure 5. Barostat for Regulating Butadiene Pressure in Absorption Cell

A comparison of the analytical results which were obtained using infrared analysis and the gravimetric maleic anhydride method is set forth in Table I.



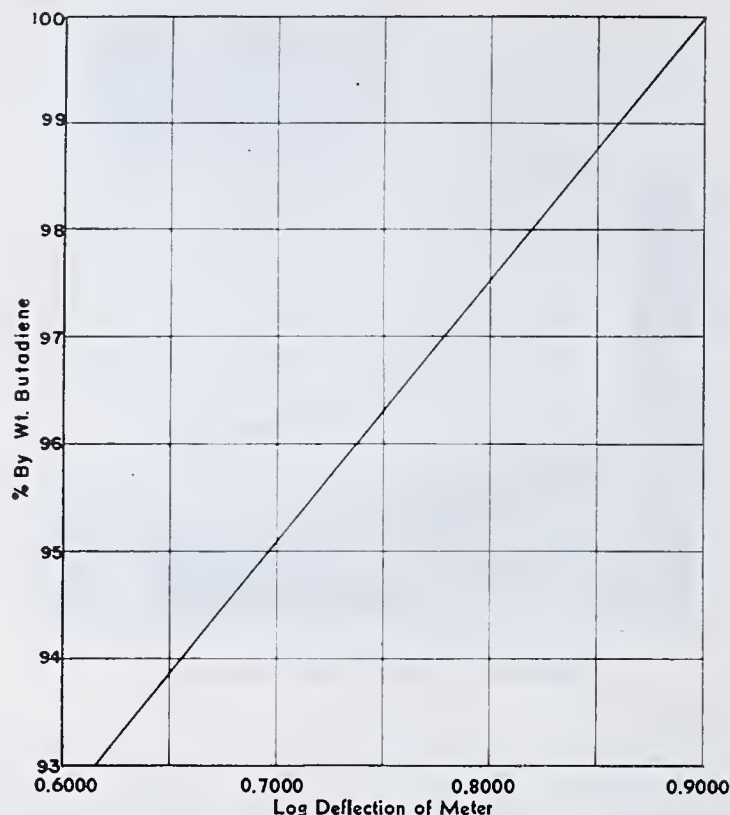


Figure 6. Work Curve for Butadiene Analysis

## DISCUSSION

Beer's law is an expression involving the number of moles present in the absorption path; consequently, an error will be introduced by substituting weight percentage for mole percentage in the work curve for butadiene. Other errors introduced through the differences in the extinction coefficients for the various impurities make it inadvisable to extend this method of analysis to butadiene concentrations below 90%. The 37 different high-purity butadiene samples shown in Table I have an average absolute deviation of 0.21% between the infrared and gravimetric maleic anhydride methods of analysis. Other studies revealed that the average absolute deviation for a number of infrared determinations made at various times by different operators was 0.04% while the gravimetric maleic anhydride method for the same series of analysis had an average absolute deviation of 0.17%.

Table I. Analyses of Butadiene by Infrared and Gravimetric Maleic Anhydride Methods

Sample	Butadiene		Sample	Butadiene	
	Infrared analysis %	Gravimetric maleic anhydride %		Infrared analysis %	Gravimetric maleic anhydride %
1	99.30	99.5	A	99.30	99.3
2	97.20	97.0	B	99.1	99.0
3	98.87	98.8	C	99.2	98.9-98.8
4	98.90	98.9	D	98.7	98.7-98.6
5	99.43	99.3	E	98.7	98.6-98.5
6	98.90	98.9	F	98.7	98.7-98.6
7	99.38	99.0	G	99.1	98.8-98.7
8	99.64	99.5	H	98.7	99.0-98.9
9	98.52	98.6	I	98.3	99.1-99.0
10	99.50	99.2	J	98.3	98.1-98.0
11	98.87	98.9	K	99.1	99.1-99.0
12	99.42	99.4	L	99.2	99.1-99.0
13	99.53	99.6	M	98.7	98.9-99.0
14	99.30	99.6	N	99.0	98.9-98.8
15	99.60	99.5	O	99.1	99.0
16	98.7	98.9-98.6	A1	99.2	98.9-98.8
17	98.7	98.7-98.7	A2	99.2	98.7
18	99.1	98.8-98.6	A3	98.7	98.7-98.6
19	98.7	99.0-98.9			

The gravimetric maleic anhydride method requires from 5 hours as compared to the 10 minutes needed to make duplicate infrared determinations. As a result of its rapidity and precision this infrared method of analysis is particularly valuable for industrial work.

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CONTRIBUTION from the Koppers Company's Fellowship on Coal Process Analysis, Mellon Institute, Pittsburgh, Pa. The procedure described in this contribution was originally evolved for and applied with success in the butadiene plant at Kobuta, Pa.

## Identification of Natural and Synthetic Rubbers

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A method is described for identification of the types of elastomers most frequently encountered in the rubber industry. The initial test depends on qualitative measurement of the pH and specific gravity of the pyrolysis products. It can be carried out in a field laboratory in 3 to 4 minutes and will provide sufficient information for a classification of the sample.

THE use of synthetic elastomers as substitutes for natural rubber has given rise to a need for a rapid method by which these materials can be distinguished from one another. The methods of identification available can be divided into two groups: those which depend on the personal judgment of the operator, such as physical appearance and the odor produced on combustion, and those which depend on detailed chemical tests. A method is proposed which is believed to be more reliable than the former, and does not require the detailed laboratory manipu-

lations necessary for a complete analysis. The method was designed for the identification of soft-rubber vulcanizates based on the polymer types represented by natural rubber, Buna S, B, N, Butyl, Neoprene GN, chloroprene-nitrile polymers, polyvinyl chloride, and polyvinyl acetate. Distinctions within types are not possible. The polysulfide rubbers are not included, as they can usually be recognized by odor.

Identifications made on the basis of physical appearance and flame tests are discussed by Kluckow (4) and Nechamkin (6). Mark (6) describes the application of some simple chemical tests such as those for nitrogen, chlorine, and sulfur. The chromic acid oxidation of natural rubber to acetic acid is described by Kuhn and L'Orsa (5); Burger, Donaldson, and Baty (2) describe a quantitative procedure based on this reaction. A method for the detection of natural rubber, which depends on the development of a purple color when the brominated product is reacted with phenol, was given by Weber (10) and was further developed by Kirchhoff (3). The method was modified



eyn and applied to the testing of synthetic rubbers (9). Experiments conducted in this laboratory indicate that Buna N can be detected by the hydrolysis of the nitrile group to an ammonium salt in the presence of sulfuric acid. The relative resistance of the hydrocarbon rubbers to the action of strong oxidizing agents has also been investigated, but the results tend to vary widely with the type of compounding.

Reliable results have been obtained by the use of a procedure which depends upon an approximate determination of the pH and specific gravity of the pyrolysis products. The tests are simple to apply and can readily be combined into a single test capable of distinguishing six of the eight types considered.

A sample of the polymer is dry-distilled under standardized conditions and a portion of the distillate collected beneath the surface of two previously prepared solutions. Solution I is yellow in color and is buffered at a pH of 4.7 with a citric acid-sodium citrate mixture. It contains thymol blue and bromothymol blue and the specific gravity is adjusted to 0.850 with methyl alcohol. Solution II is blue in color and contains sodium citrate and bromophenol blue. The pH is adjusted to 8.4 and the specific gravity to 0.890.

The color changes which the solutions undergo at different pH values are indicated in Figure 1. The cross-hatched areas show transition intervals. Table I describes the changes which take place in the two solutions when the pyrolysis products from substances considered are distilled into them. Buna N and similar materials yield ammonia and amines in sufficient quantity to produce a green color in solution I. If solution I becomes red and solution II yellow, the presence of a compound containing chlorine, such as Neoprene GN, chloroprene-nitrile, or polyvinyl chloride, is indicated. Polyvinyl acetate produces acetic acid and both solutions become yellow.

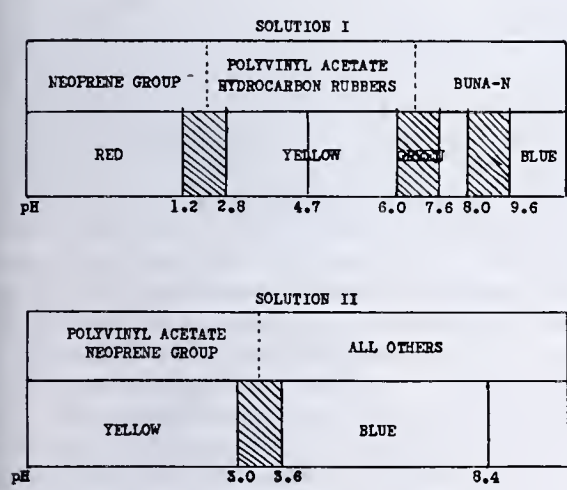


Figure 1

Natural rubber, Buna S, and butyl yield neutral products and do not materially affect the color of either solution. They are distinguished from one another by differences in the specific gravities of their pyrolysis products as indicated by their behavior in the two solutions (Table I). Table II shows some typical results obtained for the decomposition products of the three mate-

Table I. Pyrolysis Products

Rubber Type	Color of Solution		Density Behavior of Condensate	
	I	II	I	II
Buna N	Yellow	Blue	Sinks	Sinks
Neoprene GN	Green	Blue		
Chloroprene nitrile				
Polymers	Red	Yellow	Sinks	Sinks
Polyvinyl chloride				
Polyvinyl acetate	Yellow	Yellow	Sinks	Sinks
Buna S	Yellow	Blue	Sinks	Sinks
Natural rubber	Yellow	Blue	Sinks	Floats
Butyl	Yellow	Blue	Floats	Floats

In the case of natural rubber it is known that volatile hydrocarbons are formed which do not condense under the conditions of the test (?); the values obtained, therefore, depend upon a successful reproduction of the experimental conditions. The oil produced from Buna S sinks to the bottom of the test tubes in both solutions, while that from natural rubber sinks in solution I but floats in solution II. Butyl rubber produces a white, difficultly condensable vapor and a small amount of a straw-yellow oil which floats on the surface of both solutions.



Figure 2

Several additional tests are necessary for confirmatory purposes and to distinguish between substances which fall in the same group. Buna N on pyrolysis produces small quantities of cyanides which are detected by the familiar Prussian blue test. This reaction is also of value in establishing the presence of nitrile nitrogen in chloroprene-nitrile mixtures. The Beilstein test is of value for the detection of polymers containing chlorine, particularly when other polymers are present. Neoprene GN is distinguished from polyvinyl chloride by the fact that compounds based on it rapidly decolorize a solution of iodine in carbon tetrachloride. The Weber test for natural rubber (10) is of value for confirmatory purposes and for the detection of mixtures. The test may be applied directly to compounded samples without previous treatment.

Table II. Specific Gravities at 25°/4° C.

	Gum Rubber	Cured
Butyl	0.843	0.842
Natural rubber	0.860	0.873
Buna S	0.935	0.925

The rubber is brominated and heated with phenol and the melt diluted with chloroform. Natural rubber produces an opaque purple solution, Buna S a colorless to a faint purple solution, Neoprene a brown solution, and Buna N a colorless solution (9). These colors may be modified by the presence of soluble compounding ingredients, but the color produced from natural rubber is always recognizable. A colored flow chart showing the reactions obtained is useful for the interpretation of results and the instruction of plant operators.

APPARATUS

When only a few tests are to be made, the use of 16 × 150 mm. Pyrex test tubes equipped with glass condensing arms is satisfactory. A Bunsen burner adjusted to give a blue cone is a convenient source of heat.



For large-scale testing, an electrically heated furnace of the type shown in Figure 2 is desirable. The well contains a metal casting bored to accommodate three 16 × 100 mm. quartz tubes. The depth is adjusted so that 4 cm. of the length of the tubes project. The temperature of the metal block is regulated by a rheostat at 530° to 550° C. Side arms made from Pyrex tubing, 5 mm. in outside diameter, having a horizontal length of 12 cm. and a vertical length of 6 cm., are provided. After completing a test, the residue is removed from the quartz tube by ignition and the side arm cleaned with carbon tetrachloride.

#### REAGENTS

Solutions I and II are prepared according to the formulas and the specific gravities determined. They are adjusted with methyl alcohol or water to within ±0.0005 of the required value. Two-milliliter portions are placed in 10 × 75 mm. test tubes and kept tightly stoppered prior to use.

Solution I contains 0.5 gram of sodium citrate ( $2\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 11\text{H}_2\text{O}$ ), 1.00 gram of citric acid, 0.04 gram of thymol blue, and 0.10 gram of bromothymol blue dissolved in a mixture of 1000 ml. of methyl alcohol and 210 ml. of water. The pH is 4.7 at 25° C. and the specific gravity 0.850 at 25°/4° C.

Solution II contains 2.00 grams of sodium citrate and 0.01 gram of bromophenol blue dissolved in a mixture of 780 ml. of methyl alcohol and 380 ml. of water. The pH is 8.4 at 25° C. and the specific gravity 0.8900 at 25°/4° C.

The following reagents are required for carrying out the confirmatory tests: 5% ferrous sulfate solution containing 1 ml. of concentrated hydrochloric acid per 100 ml., 5% sodium hydroxide solution, 10% hydrochloric acid solution, 55% sulfuric acid solution (by weight), a solution of iodine in carbon tetrachloride containing 0.2 gram per liter, bromine, and phenol.

#### GENERAL PROCEDURE

The rubber is stripped from adhering fabric and a 1-gram sample placed in a quartz tube. A side arm is attached and the tube placed in the furnace at 530° to 550° C. After about 1 minute the sample begins to decompose. When droplets of condensate appear in the vertical section of the side arm, the end is dipped beneath the surface of 2 ml. of solution I contained in a 10 × 75 mm. test tube. After 5 to 10 seconds, or when enough oil has collected to cover the surface of the liquid, the tube is removed and the process repeated with solution II. The tubes are cooled for a few minutes until thermal equilibrium is established and then shaken sharply to determine whether the droplets formed will sink or float. The colors of the solutions and the positions of the droplets are noted and the sample is classified according to the information provided in Table I.

#### CONFIRMATORY TESTS

**GROUP I.** The presence of Buna N is confirmed by combining the contents of the tubes used in the initial test and adding 1 drop of sodium hydroxide solution, and 1 ml. of ferrous sulfate solution. The tube is heated gently for a minute and then acidified with hydrochloric acid. If a fine green precipitate is formed, the presence of nitrile nitrogen in the original sample is indicated. The suspension appears green under the conditions of the test, owing to the presence of the indicators.

**GROUP II.** The Prussian blue test described above will distinguish between Neoprene GN and chloroprene-nitrile polymers.

A 0.2-gram sample of the elastomer is shaken with 2 ml. of iodine solution. If the violet color noticeably fades in 2 to 3 minutes, the sample is Neoprene GN; if it persists, the sample is based on polyvinyl chloride.

**GROUP III.** A 0.2-gram sample is placed in a test tube together with 2 ml. of 55% sulfuric acid and warmed gently. If decomposition occurs, the compound is based on polyvinyl acetate.

**GROUP IV-V.** To detect possible interference from the presence of asphaltic extenders in the test which distinguishes natural rubber from Buna S, a 0.2-gram sample is shaken with 2 to 3 ml. of chloroform. If the chloroform darkens noticeably, the test should be repeated on a sample which has been extracted for 4 hours with chloroform (1) and dried in a vacuum oven for 1 hour at 70° C. or equivalent.

To provide a further distinction between natural rubber and Buna S, the Weber test is of value. A 0.1-gram sample is placed in a test tube and a drop of bromine added. The tube is heated gently without charring the contents and the excess bromine removed in a current of air. The sample is then covered with phenol and heated gently for a few minutes. After cooling, 10 ml. of chloroform are added. An opaque purple solution is formed if natural rubber is present. Extracted samples of

Buna S give pale violet to colorless solutions, but compound samples give solutions ranging from a light yellow to a deep brown. It has been reported that as little as 10 mg. of rubber can be detected by this reaction (9).

**GROUP VI.** The destructive distillation of Butyl rubber yields a white, difficultly condensable vapor. A light-yellow mineral oil is obtained.

#### DISCUSSION

The tests described have been applied to various materials including tires, tubes, mechanical goods, oil-resistant tubing, gas tank linings. They have been successfully used to identify reclaims made from natural rubber, Buna S, Buna N, and Neoprene GN. For routine factory testing, the initial pyrolysis procedure supplemented by the Prussian blue test is adequate. The auxiliary tests described are of value when a referee method is desired.

A complete discussion of the limitations and interferences would be too extensive for review, but a few specific examples of interest. Although the presence of citric acid and sodium citrate in the test solutions ordinarily supplies sufficient buffering action to prevent interference from minor amounts of compounds containing ingredients, the presence of large amounts of fabric in a sample may influence the result. In the case of Buna N, for instance, the initial green color reaction is not produced, and the sample may be confused with Buna S. This difficulty can be overcome by first removing the fabric or by applying the specific Prussian blue test. Buna S samples compounding with 5 parts of a diamine antioxidant were not sufficiently alkaline on pyrolysis to produce a color change. Similar compounds accelerated by 0.75 part of hexamethylene tetramine were also tested successfully.

An investigation of the reliability of the tests based on the interferences in the specific gravities of the pyrolysis products indicates that although compounds can be made up in the laboratory which do not fall within the scope of the method, they are representative of the great majority of compounds which are encountered in actual practice. An increase in the combined sulfur content tends to increase the specific gravity of the pyrolysis product obtained from natural rubber. However, samples containing 5 parts of combined sulfur can be tested successfully. Low-melting asphaltic softeners do not interfere in amounts up to 10 parts. Mineral rubber does not interfere, as the rubber composition products distill over first. In making tests on compounds of this type, it is necessary to control pyrolysis temperature carefully, and follow the details of the procedure. The portion of the distillate which comes over should be tested rather than that portion which is obtained on prolonged combustion. Interferences from extenders in amounts greater than those stated can be eliminated by a preliminary chloroform extraction, or the test can be confirmed by the use of the Weber color reaction.

The detection of mixtures has not been discussed. With many identifications can be made by the methods described, in some cases the analytical techniques involved are not readily adaptable to factory procedure.

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# Determination of Gallic Acid Added to Fats and Oils

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Methods have been developed for the quantitative determination of gallic acid added as an antioxidant to fats and oils. A spectrophotometric study of gallic acid in dilute solutions of acid and alkali is reported. The molecular extinction coefficient of gallic acid in 0.01 *N* hydrochloric acid at 270 to 271  $m\mu$  was found to be 9847. A colorimetric method based on the color produced by a ferrous tartrate reagent has been established for use with a photoelectric colorimeter.

Other methods have been developed for the quantitative determination of gallic acid and have been applied specifically to the estimation of the concentration of gallic acid added as an antioxidant to various fats and oils. The gallic acid was used as the standard and as the antioxidant in all cases. A sample of Mallinckrodt's analytical grade which was dried at 100° C. for 24 hours.

## SPECTROPHOTOMETRIC ANALYSIS FOR GALLIC ACID

Spectrophotometric measurements were made with a Bausch and Lomb Model DU spectrophotometer, with a hydrogen discharge tube as the source of the ultraviolet radiation.

A preliminary study of the absorption spectrum of gallic acid in aqueous solution showed that the position and height of the absorption peak varied with the concentration of gallic acid in solution (Figure 1). This fact suggested that ionization might be concerned, as indeed it is. In 0.01 *N* hydrochloric acid the ionization effect is suppressed and the absorption maximum is independent of the concentration.

In contrast to the colorless solution produced when gallic acid is dissolved in dilute acid, a green colored solution is produced with dilute alkali of a normality of  $2 \times 10^{-3}$ . This alkali

solution does not possess the characteristic absorption peaks which were typical of gallic acid in 0.01 *N* hydrochloric acid in distilled water, and in  $1 \times 10^{-4}$  *N* alkali. Figure 2 indicates clearly the change in position and height of the absorption maxima with the change in relative proportions of ionized and non-ionized forms of gallic acid.

Gallic acid, a weak acid, has a primary ionization constant of  $4.63 \times 10^{-5}$  for the reaction  $RH \rightleftharpoons [H^+] + [R^-]$  (1). In acid solution 0.01 *N* hydrochloric acid the ratio of  $\frac{[R^-]}{[RH] - [R^-]}$  is small, while in dilute alkali ( $1 \times 10^{-4}$  *N* potassium hydroxide) this ratio is of a larger magnitude. These two cases represent extremes in the relative ratios of ionized to non-ionized forms of gallic acid in solution. If the ratio of  $\frac{[R^-]}{[RH] - [R^-]}$  is calculated for solutions of gallic acid in distilled water as a function of concentration (Figure 1) the experimentally observed shifts will follow the change in calculated ratios—i.e., the greater the ratio the greater the shift in maximum toward a shorter wave length.

The absorption curve for gallic acid in 0.01 *N* hydrochloric acid was measured and showed a minimum at 238 to 239  $m\mu$  and a maximum at 270 to 271  $m\mu$  (Figure 2). When aliquots of a standard solution of gallic acid were diluted to different concentrations in 0.01 *N* hydrochloric acid, the optical densities at 270  $m\mu$  of the various dilutions varied in direct proportion with their concentration in the range from 0.005 to 0.016 mg. per ml. The molecular extinction coefficient was calculated to be 9847 on the basis of a molecular weight of 170.1 for gallic acid by the equation:

$$\epsilon = \frac{d}{CL}$$

where

$$d = \text{optical density} = \frac{\log \text{intensity of incident light } (I_0)}{\log \text{intensity of transmitted light } (I)}$$

$$C = \text{concentration in moles per liter}$$

$$L = \text{thickness of the solution in cm.}$$

Hertzówna and Marchlewski (3) found an absorption minimum at 238  $m\mu$  and an absorption maximum at 265  $m\mu$  for gallic acid in aqueous solution. The concentrations of gallic acid were  $2 \times 10^{-4}$  and  $1 \times 10^{-4}$  mole per liter. From the magnitude of the calculated ratio of  $\frac{[R^-]}{[RH] - [R^-]}$  for these two solutions one would predict the absorption maximum in the region of 264 to 265  $m\mu$ .

## COLORIMETRIC ANALYSIS FOR GALLIC ACID

A colorimetric method for the determination of gallic acid has been developed, based on the color developed by ferrous tartrate in the presence of polyphenolic compounds (2, 4). With certain modifications it should be applicable to polyphenolic compounds other than gallic acid.

Two milliliters of the freshly prepared color reagent (0.5% sodium potassium tartrate and 0.1% ferrous sulfate) were added to an aqueous solution of the gallic acid sample in a 100-ml. volumetric flask. A violet color developed when the solution was buffered to pH 7 by the addition of 10 ml. of a 10% solution of ammonium acetate and sufficient distilled water to adjust the final volume of the reaction mixture to 100 ml.

When the absorption curve for the violet solution was measured in the Beckmann spectrophotometer, it was found to have an absorption maximum at 540  $m\mu$  (Figure 3).

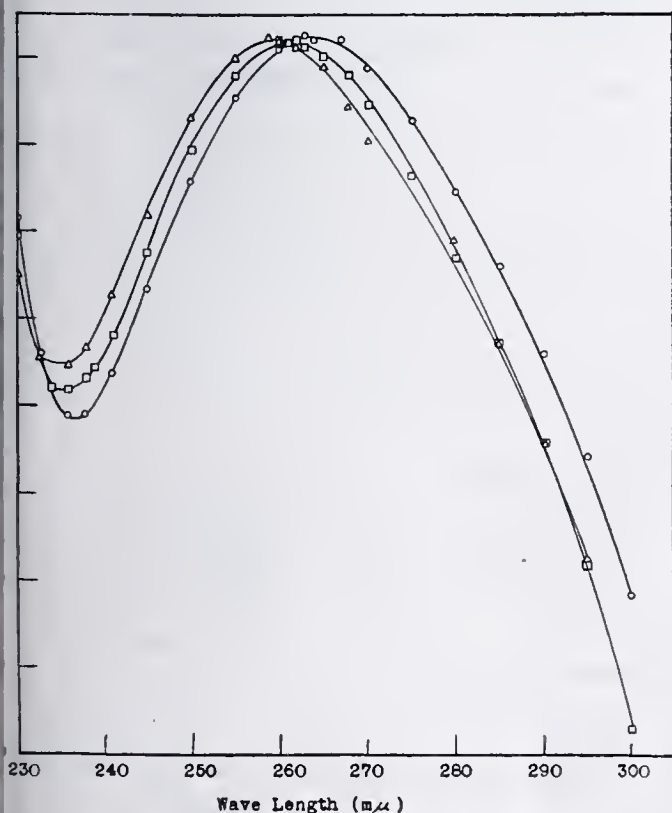


Figure 1. Spectral Curve for Gallic Acid in Distilled Water  
 $7.6 \times 10^{-5}$  mole per liter.  $\square$   $5.88 \times 10^{-5}$  mole per liter.  $\triangle$   $3.53 \times 10^{-5}$  mole per liter



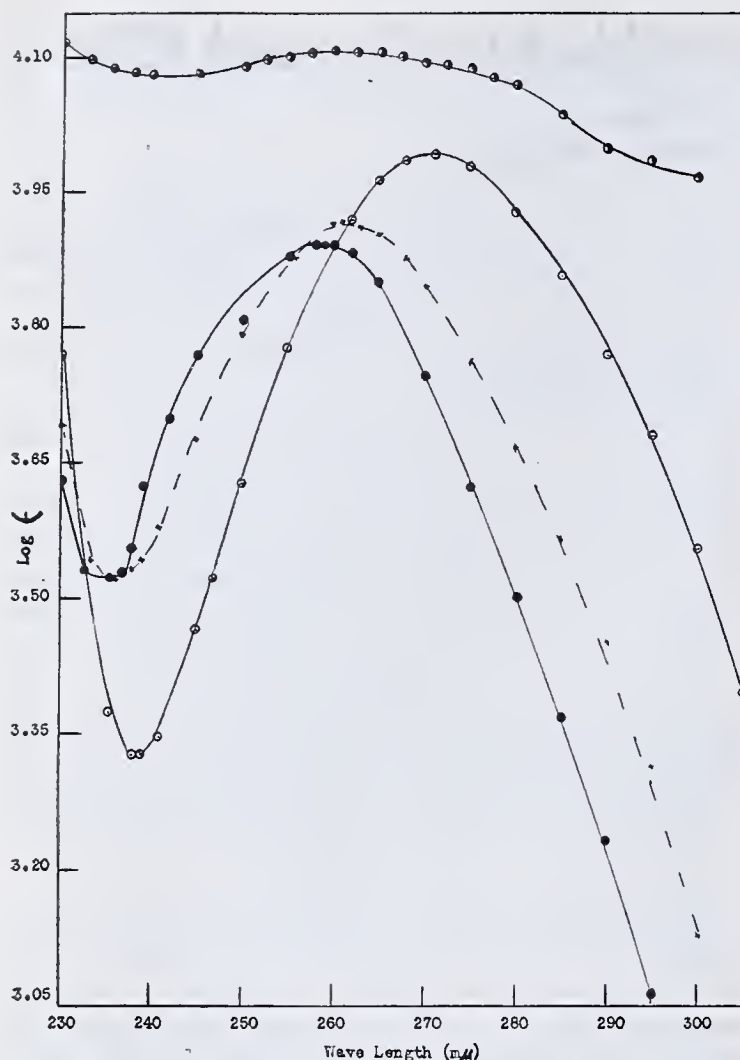


Figure 2. Spectral Curves for Gallic Acid in Dilute Solution of Acid and Alkali

Concentration of gallic acid,  $5.88 \times 10^{-5}$  mole per liter.  $\circ$   $1 \times 10^{-2}$  N HCl.  $\times$  Distilled water.  $\bullet$   $1 \times 10^{-4}$  N KOH.  $\bullet$   $2 \times 10^{-3}$  N KOH

In order to measure quantitatively the depth of the violet color, samples of various dilutions were examined in an Evelyn photoelectric colorimeter, with a 540 mμ filter. It was found that a linear relationship existed between the concentrations of gallic acid used to develop the color and the intensity of the color as measured by the  $L$  readings ( $\log 100 - \log$  galvanometer reading for the sample). The concentrations of gallic acid employed in the standardization were between 0.2 and 1.0 mg. per 100 ml. of solution. The blank contained only the color reagent and the buffer. The slope of the resulting best straight line was calculated by the method of least squares to be  $0.325 \pm 0.006$ . This slope represents the  $K$  value to be used in the calculation of gallic acid concentrations in experimental samples from the equation:

$$C = \frac{L}{K}$$

where

$L = \log 100 - \log G$  (where  $G$  is the galvanometer reading of the sample obtained under conditions such that the galvanometer reading for the reagent blank was 100)  
 $C =$  concentration in mg. of gallic acid per 100 ml. of solution.

The violet color developed rapidly and was found to be stable for several hours. Addition of twice the specified amount of color reagent did not increase the intensity of the color produced, nor did the addition of a drop of concentrated ammonium hydroxide affect the color. These facts provide a satisfactory degree of flexibility in the application of the method.

#### APPLICATION OF METHODS TO ESTIMATION OF GALLIC ACID ADDED AS AN ANTIOXIDANT TO FATS AND OILS

In order to remove the gallic acid from a fat sample, a weighed amount (5 to 10 grams) of the fat or oil was extracted by adding 65 ml. of distilled water and bringing the mixture to a boil. The mixture was set aside to cool and then filtered into a 100-ml. volumetric flask through a water-wet filter paper which allowed

the aqueous solution to pass but retained the oil. The original flask and the filter paper were washed with an additional 20 ml. of distilled water in divided portions (5 ml.), and then the combined extract and washings were ready for analysis by either of the above methods. For the spectrophotometric method, the combined extract and washings were made to volume (100 ml.) before analysis. In the colorimetric method, however, the color reagent (2 ml.) and buffer solution (10 ml.) were added before the resulting solution was made to volume (100 ml.). If the concentration of gallic acid was too high (1 mg. per 100 ml. in the colorimetric and 1.6 mg. per 100 ml. in the spectrophotometric method) to be within the range of either method, the solution was made to volume and suitable aliquots were taken for analysis.

The recoveries by both spectrophotometric and colorimetric methods, when known amounts of gallic acid were added to samples of cottonseed oil, are given in Table I. Gallic acid is not appreciably soluble in cottonseed oil and each sample tested was really a suspension of gallic acid. This fact limited the

Table I. Recoveries of Gallic Acid

Added Mg.	Colorimetric Found Mg.	Added Mg.	Spectrophotometric Found Mg.
0.40	0.37 0.38	0.50 0.25 0.20	0.49 0.27 0.21
0.60	0.59 0.59	0.15 0.10	0.15 0.10
0.80	0.78 0.80	Average of duplicate analyses.	

Table II. Results on Unknown Experimental Samples

Sample	Concentration of Gallic Acid Spectrophotometric method Mg./g.	Colorimetric method Mg./g.
1	0.265	0.263
2	0.120	0.110
3	0.0025	0.0033

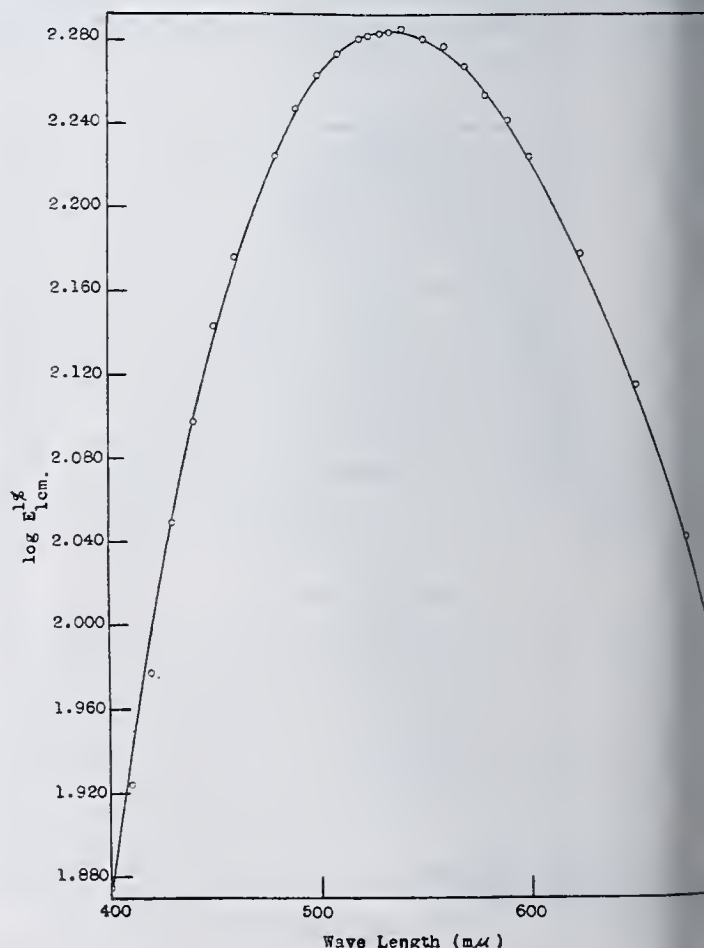


Figure 3. Absorption Curve of Colored Complex Formed by Reaction of Gallic Acid with Ferrous Tartarate at pH 7



of duplicate analyses beyond the ordinary tolerance of spectrophotometric or colorimetric analyses. In Table II are obtained by both methods on some unknown experimental samples.

#### SUMMARY

Spectrophotometric method and a colorimetric method have been developed for the quantitative determination of gallic acid. The precision of the ionization of gallic acid in the former method is satisfactory. The latter method may be applied to other polyphenolic compounds.

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# Photometric Method for Determination of Hemicellulose

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The first step in the production of viscose rayon is the steeping of the pulp in 18% sodium hydroxide solution to convert the cellulose to soda cellulose and to remove the greater part of the alkali-soluble material contained in the pulp. This alkali-soluble material is usually referred to as hemicellulose. According to Heuser (1) for sulfite pulp, it consists principally of degraded cellulose, with some xylan and mannan. A constant and relatively low hemicellulose concentration in the steeping solution is required, necessitating the use of a recovery system in economical plant operations. In order to assure the necessary close control of hemicellulose concentration, a number of determinations must be made daily in a viscose plant.

Various methods used in the Industrial Rayon laboratories for the oxidation of the hemicellulose with chromic acid-sulfuric acid mixtures by boiling for different lengths of time, either with or without a reflux condenser, and determining the excess of dichromate iodometrically. These methods give reproducible results but are time-consuming and require the use of a rather expensive chemical, potassium iodide. The reduction of orange chromic acid to green chromic sulfate results in a mixture of products not suitable for accurate colorimetric determinations, but the extent of the reduction can be easily determined with a spectrophotometer or filter photometer.

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#### EXPERIMENTAL

According to Jäger (2) it is necessary to maintain a temperature of 125° to 135° C. for at least 5 minutes in order to complete the oxidation of hemicellulose and glucose with a chromic acid-sulfuric acid mixture. However, one of the authors found that in the temperature range 130° to 140° C. the reaction is essentially complete in one minute or less—further heating changes the results so little that, for control work, the additional time required is considered unjustified. The authors' method assumes that the composition of the dissolved hemicellulose is the same at all times, since a change in composition would result in a different reducing power per unit weight of hemicellulose. Uniformity in the composition of the hemicellulose is assured by the high degree of uniformity of the pulp supplied to rayon manufacturers.

A wide range of hemicellulose concentrations is encountered in these laboratories. In order to obtain high sensitivity and still keep the method simple as possible, the same amount of *N* potassium dichromate solution, 5.0 ml., is mixed with varying amounts of hemicellulose solution, according to the concentration present. The following table shows the amount of sample used for the different hemicellulose concentrations:

Concentration Range, %	Ml. of Sample
0.00-0.12	25.0
0.12-0.30	10.0
0.30-1.20	2.0
1.2-2.4	1.0

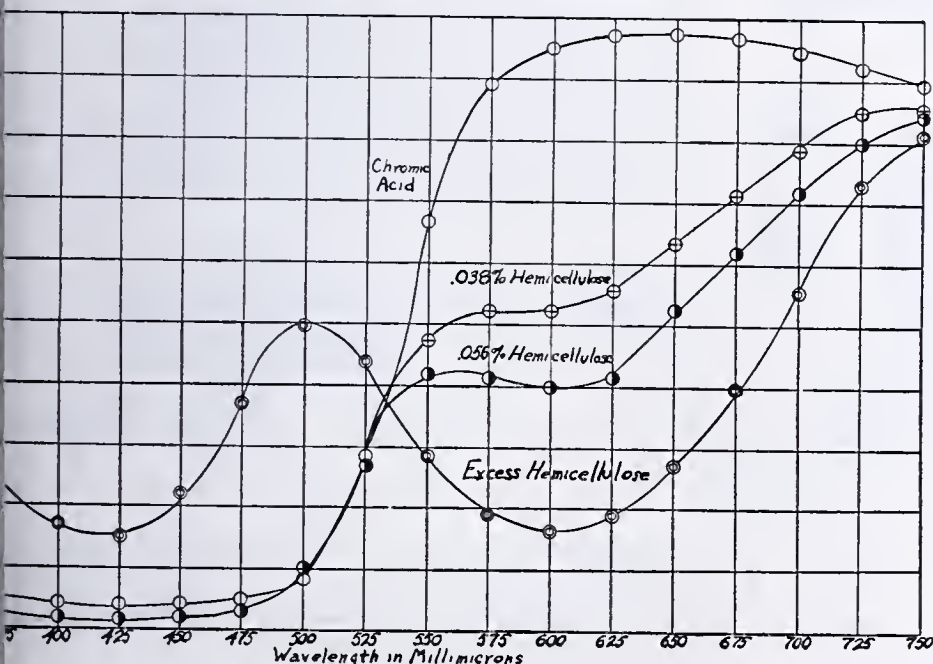


Figure 1. Transmittance Curves for Chromic Acid-Hemicellulose Solutions

For samples less than 25.0 ml., sufficient c.p. sodium hydroxide solution or distilled water is added to bring the volume to 25.0 ml. Small variations in the sodium hydroxide concentration of the solutions do not affect the results appreciably, but the sodium hydroxide concentration should be approximately the same as in the hemicellulose solutions used in preparing the calibration curves. Finally, 25 ml. of concentrated sulfuric acid are added cautiously to the mixture of potassium dichromate and sodium hydroxide solutions in a 250-ml. Erlenmeyer flask. The solution is boiled for about 30 seconds and then cooled. The final volume is  $49 \pm 1$  ml. The solutions thus prepared are stable, showing no change in transmittance at 600 millimicrons after standing for months in glass bottles.

All transmittance measurements were made with a Coleman Universal spectrophotometer. The solution cuvettes have a thickness of 13 mm. The wave band passing through the exit slit is 35 millimicrons wide. Distilled water is



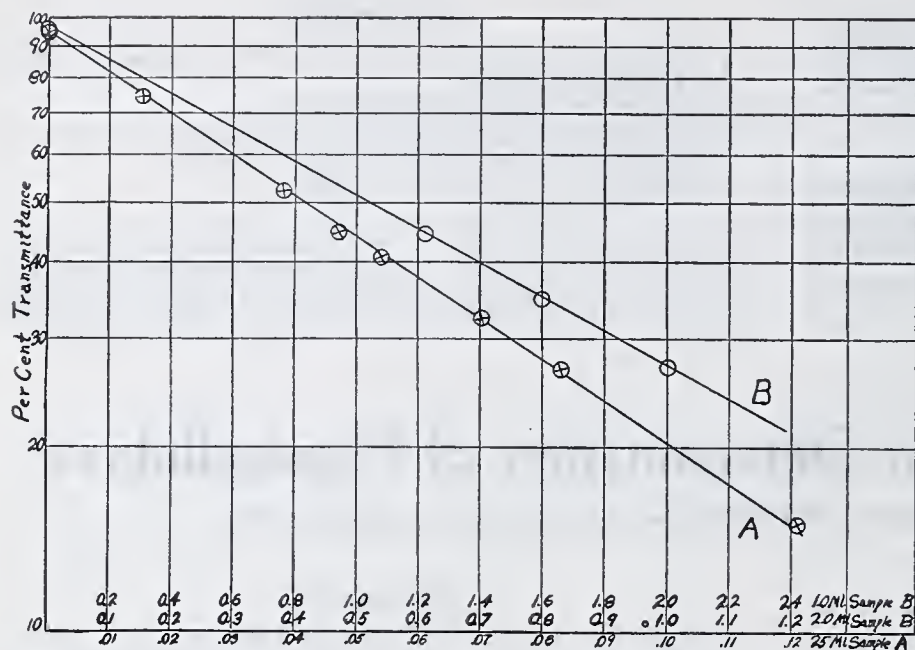


Figure 2. Per Cent Hemicellulose in Sodium Hydroxide Solutions

used in the reference cell at all times and the transmittance is measured to the nearest 0.25%.

Figure 1 shows transmittance measurements for solutions prepared as above, using 25.0-ml. samples of sodium hydroxide solutions containing varying amounts of hemicellulose. The curves show that the maximum spread between the transmittance of the chromic acid and chromic sulfate occurs at 600 and 625 millimicrons. The former wave length was chosen for transmittance measurements because an error in the wave-length setting of the spectrophotometer will result in a minimum error in the transmittance of the solution. If a photoelectric filter photometer is employed for measurements with these solutions, a red filter should be used.

Calibration curves were prepared, following the procedure outlined above, measuring the transmittance of the cooled solution at 600 millimicrons, and then determining the hemicellulose in the same solution iodometrically. The hemicellulose concentration is calculated using the factor 1 ml. of *N* potassium dichromate = 0.00675 gram of hemicellulose (as glucose). The conditions assumed in the calculation of this factor—i.e., only pure cellulose present and complete oxidation to carbon dioxide and water—are not realized in the authors' work, so no claim is made concerning the accuracy of the determinations. The results are shown in Figure 2, plotted on a semilogarithmic scale. The concentration of hemicellulose is on a weight basis, although the solutions were measured by means of pipets. The curves show that Beer's law holds up to a hemicellulose concentration at which nearly all the chromic acid is reduced. This simplifies the preparation of calibration curves. For routine use, charts were prepared from the calibration curves. The precision under routine conditions is approximately  $\pm 1.5\%$ , but this can be improved by carefully reproducing experimental conditions. The principal source of error in the method as described is in the evaporation loss. It is necessary to control the boiling time closely.

In a modification developed to permit the use of a larger sample for the higher

hemicellulose concentrations, 25 ml. of *N* potassium dichromate are mixed with 5.0 ml. hemicellulose solution in a 250-ml. Pyrex volumetric flask, and 25 ml. of concentrated sulfuric acid are added cautiously. The solution is heated to boiling, cooled, and diluted to mark with distilled water, and the transmittance of the well-mixed solution at 600 millimicrons is measured.

This method, in addition to the advantage of the larger sample, also eliminates the volume error. Dilution to a definite volume can be used with the first method described. However, a rather slow change in the color of the solution takes place after dilution. Figure 3 shows the change in transmittance of a solution that contained an excess of hemicellulose. The change is very nearly complete in 30 minutes after dilution. It seems to be due to the reduction in concentration of sulfuric acid, so little change occurs when the solution is diluted with 1 to 1 sulfuric acid instead of water.

#### SUMMARY

A quick photometric method for the determination of organic material dissolved in sodium hydroxide solutions from wood pulp is presented. A Coleman Universal spectrophotometer was used in developing the method, but it has also been used with two different photoelectric filter photometers. The method is well suited to routine use and determinations can be completed in 5 minutes. The authors believe that the method can be adapted for the quick determination of a variety of organic materials dissolved or suspended in an aqueous medium.

#### ACKNOWLEDGMENTS

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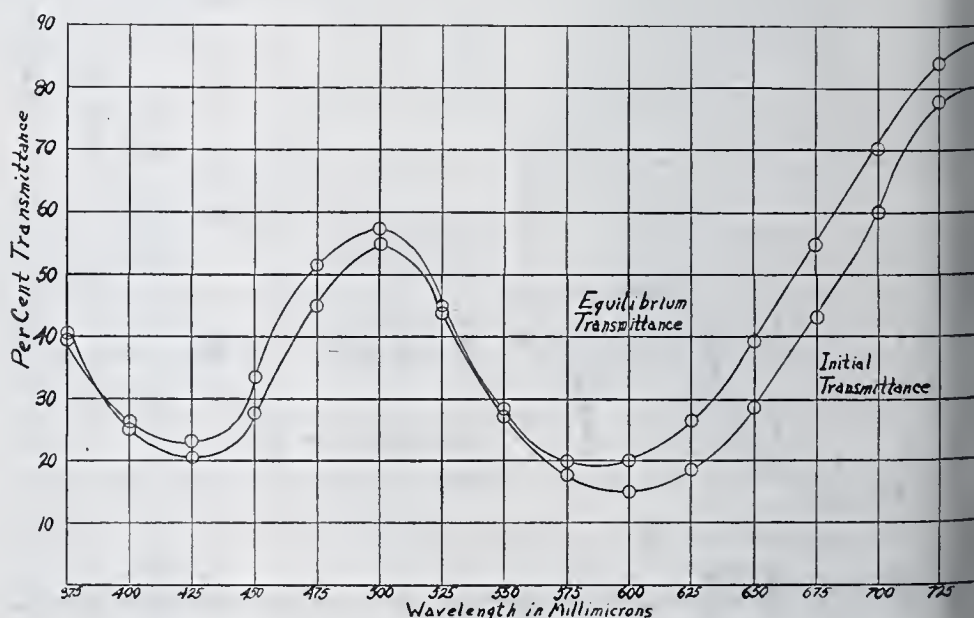


Figure 3. Change in Transmittance with Time

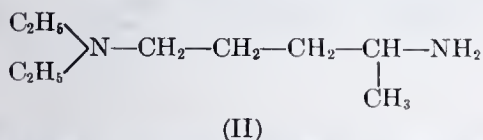
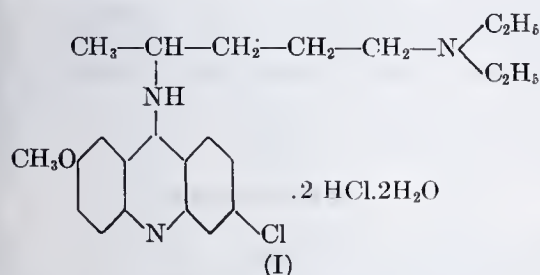


# Purification and Gravimetric Determination of 1-Diethylamino-4-aminopentane

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venient method for preparing pure 1-diethylamino-4-amino-  
ne is described. A simple and accurate gravimetric deter-  
tion of 1-diethylamino-4-aminopentane has been developed,  
l upon the quantitative precipitation of the amine as its dithio-  
mate from acetone solution with carbon disulfide.

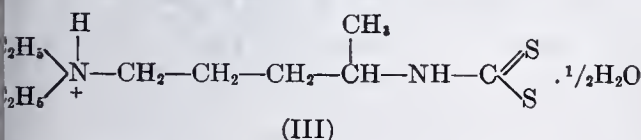
CONNECTION with the manufacture of quinacrine hydro-  
chloride (I) it was necessary to have a reliable method for  
izing the amino side chain, 1-diethylamino-4-aminopentane



mmercial 1-diethylamino-4-aminopentane from five differ-  
ources was found to range in purity from 70 to 96%. Among  
ururities present were other aliphatic amines, and, for this  
a, a simple titration with acid was of no analytical value.  
eal measurements such as refractive index, density, and  
g range were also found to be of little use. The conven-  
Van Slyke amino nitrogen assay has been applied to 1-  
ylamino-4-aminopentane and is said to give satisfactory  
s, but it is specific only for primary amines. Obviously, if  
ther primary amines were present as impurities in 1-di-  
amino-4-aminopentane, the Van Slyke assay would give  
eous results.

1-diethylamino-4-aminopentane reacts with carbon disulfide  
rm an unusually stable and insoluble dithiocarbamate.  
rystalline compound has proved to be an excellent deriva-  
or both the purification and the gravimetric determination  
e amine. The precipitation of the dithiocarbamate from  
us acetone solution containing about 2 or 3% water is  
eally quantitative, and at the same time it effects a sharp  
tion of 1-diethylamino-4-aminopentane from all the impu-  
which have been encountered so far in the commercial prod-

en it is formed in the presence of water, the dithiocarbamate  
lizes with 0.5 molecule of water. *Analysis.* Calculated  
H<sub>22</sub>N<sub>2</sub>S<sub>2</sub>.1/2H<sub>2</sub>O: N, 11.5. Found: N, 11.5. It is prob-  
est represented as an intramolecular salt (III) (3):



en formed in absolute alcohol, the dithiocarbamate con-  
one-half molecule of alcohol of crystallization. *Analysis.*  
lated for C<sub>10</sub>H<sub>22</sub>N<sub>2</sub>S<sub>2</sub>.1/2C<sub>2</sub>H<sub>5</sub>OH: N, 10.9. Found: N,  
11.0. The dithiocarbamate without any solvent of crys-  
tation is obtained when the pure amine in dry, alcohol-free  
solution is treated with carbon disulfide. All three forms

melt with decomposition at about 136–138° C. (uncorrected).  
Both the hemialcoholate and the anhydrous compound are  
quickly converted to the hemihydrate upon treatment with wa-  
ter. The hemihydrate is soluble in water to the extent of 1.175  
grams per 100 ml. and in 95% alcohol to the extent of 0.048 gram  
per 100 ml. at 30° C. In acetone, ether, carbon disulfide, or ben-  
zene the solubility is extremely small. Aqueous alkali solutions  
readily dissolve the dithiocarbamate, and acids decompose it with  
the liberation of carbon disulfide.

For the purification of 1-diethylamino-4-aminopentane the  
dithiocarbamate was best formed by adding the crude amine  
slowly and with cooling and stirring to an excess of carbon disul-  
fide in about 3 volumes of 95% alcohol, or acetone containing 5 to  
10% water. The compound was washed with a little acetone,  
and then treated with an excess of concentrated hydrochloric  
acid. After the liberated carbon disulfide was removed, the  
amine hydrochloride was converted to the free amine in the  
usual way to give a product with a boiling range of 0.5° to 1°.

Pure 1-diethylamino-4-aminopentane obtained by this pro-  
cedure had the following constants: boiling point, 200–200.5° C.  
(753 mm.);  $n_D^{25}$ , 1.4403 (temperature coefficient = 0.00045 per  
degree);  $d_{25}^{20}$ , 0.819. These constants did not change upon re-  
peated reprecipitation of the amine as the dithiocarbamate, fol-  
lowed by reconversion back to the free amine.

A number of other amines form precipitates with carbon  
disulfide in acetone solution, and if any of them were present  
as impurities in 1-diethylamino-4-aminopentane they might  
interfere with its purification and gravimetric determination.  
Some of these other substances are ammonia, piperidine, ethyl-  
enediamine (1), *N,N*-diethylethylenediamine (2), 2,3-diamino-  
butane (4), 1-diethylamino-3-aminopropane, 1-diethylamino-4-  
aminobutane, 1-diethylamino-5-aminopentane, putrescine, and  
cadaverine (3). Presumably all diamines structurally similar to  
1-diethylamino-4-aminopentane will form acetone-insoluble di-  
thiocarbamates. On the other hand, none of the common ali-  
phatic monoamines such as methylamine, ethylamine, diethyl-  
amine, ethanolamine, or any tertiary amines are precipitated  
from acetone solution by carbon disulfide. Fortunately, we  
have not found in commercial 1-diethylamino-4-aminopentane  
any of these other amines which might interfere with its purifi-  
cation or analysis by the dithiocarbamate method.

Table I. Determinations Using Pure Amine

		(99.72% by acid titration)					
Weight of Sample Gram	Theoretical Grams	Weight of Precipitate					
		2 Hours Grams	4 Hours Grams	16 Hours Grams	25 Hours Grams	46 Hours Grams	88 Hours Grams
0.8135	1.2475	1.2630	1.2595	1.5315	1.5317	1.2522	...
0.9981	1.5305	...	...	1.5315	1.5317	1.5297	...
0.8414	1.2900	...	...	1.2866	1.2866	1.2847	1.2807
0.8798	1.3491	...	...	1.3458	1.3458	1.3437	1.3398
0.6925	1.0622	...	...	1.0569	1.0567	1.0548	1.0527

Table II. Analyses of Known Mixtures

		(Precipitates dried 16 hours)				
		Composition				
No.		$\beta$ -Diethyl- amino- ethanol %	1-Diethyl- amino- pentanol-4 %	1-Diethyl- amino- pentanone-4 %	1-Diethyl- amino- 4-amino- pentane %	1-Diethylamino- 4-aminopentane Found %
1	...	...	...	...	99.72	99.5, 99.5, 99.3, 99.8, 100.0
2	20.3	5.1	5.0	69.6	69.9	69.9, 69.9, 69.4, 69.8
3	11.6	5.0	5.1	78.3	78.7	78.7
4	...	4.9	5.2	89.9	89.5	89.5, 90.0, 89.5, 89.9
5	10.4	...	...	89.6	90.0	90.0
6	80.4	4.6	4.8	10.2	8.5	8.5, 9.4



Table III. Typical Analyses of Commercial 1-Diethylamino-4-aminopentane

(Precipitates dried 16 hours)			
Sample	Found, %	Sample	Found, %
A	84.1, 83.6, 83.7	E	78.1, 78.1, 77.9
B	96.8, 96.5, 96.7	F	96.3, 96.3, 96.6
C	80.7, 80.5	G	83.8, 83.8, 83.9
D	86.2, 86.2	H	81.5, 81.0, 81.8

The gravimetric determination of 1-diethylamino-4-aminopentane consists of precipitating it as the dithiocarbamate from aqueous acetone solution containing about 2% water, collecting the precipitate on a tared filtering crucible, washing with acetone, and drying in vacuum over calcium chloride. The acetone used in the following procedure was dried over potassium carbonate and filtered.

**PROCEDURE.** About 1 gram of the amine sample was weighed to the nearest 0.1 mg. into a 100-ml. beaker. Without delay, 20 ml. of acetone were added by washing down the sides of the beaker, and this was followed by 0.75 ml. (15 drops) of water. To the resulting solution were added 10 ml. of a 15% (by volume) solution of carbon disulfide in acetone. The mixture turned light brown, and after a few seconds a white crystalline precipitate began to appear. The mixture was stirred with a small glass rod for about one minute or until precipitation was largely complete, and then it was allowed to stand. After 0.5 hour, 25 ml. of acetone were added, and the mixture was well stirred and allowed to stand for an additional 0.5 hour.

The precipitate was loosened from the walls of the beaker with a rubber-tipped rod, and the acetone solution was decanted through a tared filtering crucible. The precipitate was washed by decantation with two 10 to 15-ml. portions of acetone and then transferred to the crucible with acetone.

After the precipitate had been sucked as dry as possible the crucible was placed over anhydrous calcium chloride in a vacuum desiccator which was evacuated to 5 to 10 mm. and allowed to stand at room temperature. The precipitate was weighed after drying for at least 5 hours and preferably longer.

As obtained by the above procedure, the precipitate has the formula  $C_{10}H_{22}N_2S_2 \cdot \frac{1}{2}H_2O$ . The effect of the length of drying the precipitate in vacuum over calcium chloride is indicated in Table I. The weights remain constant between 16 and 25 hours, but at 46 hours some loss becomes apparent. In determinations on commercial samples, the weight became most constant after drying about 5 to 8 hours. The optimum time for drying is, therefore, probably between 5 and 46 hours.

Analyses of mixtures containing known quantities of 1-diethylamino-4-aminopentane are presented in Table II. The compounds used in the mixtures,  $\beta$ -diethylaminoethanol and 1-diethylaminopentanone-4, are commonly present as impurities in 1-diethylamino-4-aminopentane. The analysis is satisfactory only in the case (No. 6) in which 1-diethylaminopentane is a minor constituent of the mixture.

In Table III are listed some typical analyses on commercial samples of 1-diethylamino-4-aminopentane. The agreement between duplicate and triplicate determinations is considered satisfactory for a method of this kind.

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## Analysis of Solutions of Ethyl Ether, Benzene, Ethyl Alcohol, and Water

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A method for the analysis of mixtures of ethyl ether, benzene, ethyl alcohol, and water has been developed. It has been applied to three synthetic mixtures containing 1 to 15% ethyl ether, 1 to 30% benzene, 53 to 84% ethyl alcohol, and 7 to 15% water and shown to yield results which, when expressed as per cent of a component present, have an accuracy and a precision of about 1%.

**V**ARIOUS methods are in use for the determination of ethyl ether, benzene, and ethyl alcohol individually in the presence of water. Although Masson and McEwan (10), Desmaroux (4), and Kubias (7) studied ternary mixtures of certain of these components with water, no rapid procedure has been available for the complete analysis of quaternary mixtures of water and appreciable quantities of each of these solvents. The method described in this paper was developed for control purposes only and cannot be used for the analysis of mixtures of unknown composition.

In the following method the ether is driven off and determined by loss in weight; the benzene is determined by measuring (a) the water-insoluble portion of the residue and (b) the water-immiscible portion that remains in a distillate from this ether-free residue after removing the alcohol from the distillate by oxidation. Alcohol and water are determined on original samples—alcohol by acetylation and water by the Karl Fischer method. Any one of the four components not present in a small quantity, can be satisfactorily determined by the difference between 100% and

the sum of the other three components. The method is a simple one which requires no expensive equipment.

Benzene may also be determined on an original sample by the recently published colorimetric methods of Dolin (8) and Baernstein (3), which were not available at the time the method described in this paper was being developed.

#### DETERMINATION OF ETHER

Preliminary experiments showed that ether could not be separated easily and sharply from benzene and alcohol by distillation through a simple column having a minimum holdup. However, it can be separated well from the other components if the sample is first treated with an equal volume of water, regardless of the fact that a two-phase system may thus be formed.

**PROCEDURE.** A sample of approximately 100 ml. is weighed in a 300-ml. round-bottomed flask containing a few particles of Carborundum, about 100 ml. of water are added to the flask, the whole is weighed again. The flask is attached to a 3-ft. Snyder column (13) as indicated in Figure 1. A rather strong current of air is directed against the lower bulb of the column to ensure adequate refluxing, then the flask is heated gently with a flame until the distillation starts. The precautions which must be observed in this operation are shown in Figure 1. The heating must be conducted cautiously in case there are two liquid layers in the flask. The flask is heated until the thermometer shows a vapor temperature of 52° C., and the temperature is maintained as close as possible to 52° for 3 minutes. The column is allowed to drain for 1 minute; the flask is removed and weighed again. The decrease in weight is the weight of ether in the sample.



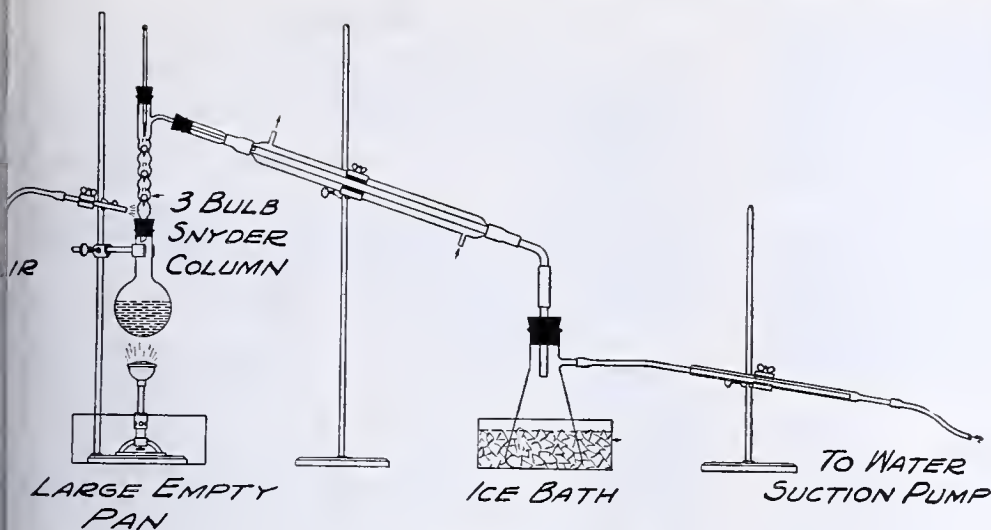


Figure 1. Diagram of Apparatus

The distillation is continued until a volume of 20 to 25 ml. has been collected in an Eggertz tube or in a 50-ml. buret at the rate of 1.0 to 1.5 ml. a minute. Fifteen milliliters of 3% potassium dichromate solution and 2 ml. of concentrated hydrochloric acid are added to the distillate. The mixture is shaken vigorously and allowed to stand for at least 15 minutes. Exactly 10.0 ml. of gasoline or naphtha are added with an accurate pipet or buret. The mixture is again shaken and allowed to stand until the layers separate. The volume of the upper layer is read. This volume, less 10.0 ml., is the volume of benzene in the benzene-alcohol-water distillate. From the total volume of benzene found in the two fractions, the per cent of benzene in the sample is calculated. To facilitate this calculation, it may be stated that the density of benzene varies from 0.878 at 20° to 0.868 at 30°.

#### DETERMINATION OF ALCOHOL

The original sample is analyzed for alcohol by the usual pyridine acetylation method (12, 15). In applying this method, particular attention must be given to the size of the samples taken. If more than 30% water is present, it is advisable to determine the alcohol by some other method. To make this reaction proceed to the maximum extent, a large excess (at least 300%) of acetic anhydride is required.

The acetylation of ethyl alcohol has been found to be 99.1% complete when acetyl chloride is used as the reagent (14), and Moore and Blank (11) recently found that glycerol is acetylated to the extent of 99.3% by acetic anhydride in pyridine. Under the usual analytical conditions of using acetic anhydride in pyridine, the degree of acetylation of two specimens of 95%-bicy volume ethyl alcohol was determined. Assuming that the alcohol contained no appreciable impurity other than water, the results in Table I show acetylation to 98% of the stoichiometric value; therefore a correction factor of 1.02 was applied in calculating the results for ethyl alcohol in the quaternary mixtures reported in Table II.

**PROCEDURE.** In preparing 2.4 *N* acetylation reagent, pyridine containing about 0.35% water is used to prevent the resin formation that would otherwise occur when the reagent is heated (16). Malm, Genung, and Williams (9) have shown that the addition of water is unnecessary when the reaction is conducted at a lower temperature and with a less concentrated reagent. Twenty-five-milliliter portions of reagent are placed in each of a number of Erlenmeyer flasks. Assuming the water content of the material analyzed to be 15% or less, samples of approximately 0.8 gram are weighed directly into the reagent from a Smith weighing buret. If a greater proportion of water is present, the sample must be correspondingly smaller. It is helpful to place a few particles of Carborundum in each flask.

The flasks containing reagent only, for blank determinations, and those containing reagent plus samples are attached to water condensers whose inner surfaces are dry and refluxed for at least

Table I. Acetylation of Ethyl Alcohol

Specimen No.	Water, by Karl Fischer Method %	Alcohol Present, by Difference %	Alcohol Found, by Acetylation %	Extent of Acetylation of Alcohol %
1	7.22		90.77	
	7.24		90.79	
	Av. 7.23	92.77	90.78	97.9
2	7.80		90.45	
	7.70		90.59	
	Av. 7.75	92.25	90.61	98.2
				Av. 98.1

#### DETERMINATION OF BENZENE

When a considerable proportion of benzene is present, it has been found advisable to determine it by measuring (a) the water-soluble portion of the residue from the ether distillation and (b) the fraction of the water-soluble portion of a distillate from the residue that is not oxidized by 3% potassium dichromate and concentrated hydrochloric acid. These reagents oxidize the alcohol in the distillate. This procedure was developed by Babton and Tingle (2). In case only a little benzene is present, there will be no water-insoluble portion in the residue from the ether distillation, and the determination will consist only of measuring fraction b—i.e., the benzene dissolved in the residue.

**PROCEDURE.** The residue left after the distillation of ether is cooled. If this cooled residue consists of two layers, they are separated and the benzene is determined in each of them separately as follows: The contents of the flask are poured into a 250-ml. separatory funnel, and 10 ml. of saturated sodium chloride solution are added. The flask need not be rinsed. The mixture is shaken vigorously. The lower layer is transferred to the distillation flask previously used.

The upper layer in the separatory funnel is washed twice with an approximately equal amount of water, and the wash water is transferred each time to the distillation flask. This treatment removes alcohol from the benzene. The benzene is then transferred either to a dry 50-ml. Eggertz tube or to a buret which has previously been filled with benzene to the lowest graduation mark. The volume of the benzene is read. The separatory funnel is rinsed with benzene-free ethyl alcohol into the distillation flask which contains water, alcohol, and a small amount of dissolved benzene. The flask is attached to the Snyder column and condenser, and the distillation is heated gently, so that the distillation will start slowly. An air current is not directed against the lower end of the column in this distillation.

Table II. Analysis of Synthetic Mixtures

Synthetic Mixture No.	Analysis No.	Ether		Benzene		Alcohol		Water		Total
		Present %	Found %	Present %	Found %	Present %	Found %	Present %	Found %	
1	1	1.5	1.6	30.2	29.9	52.7	53.2	15.6	15.7	100.4
	2		1.3		29.5		54.4		15.8	
	3		1.5		..		53.5		15.4	
	4		..		..		53.1		..	
	Av.		1.5		29.7		53.6		15.6	
2	1	14.8	15.1	14.7	14.9	56.3	56.8	14.2	14.3	99.8
	2		14.1		15.3		55.2		14.2	
	3		14.4		14.6		56.3		14.3	
	Av.		14.5		14.9		56.1		14.3	
3	1	7.5	8.0	1.0	0.9	84.3	84.9	7.2	7.1	101.3
	2		7.9		0.9		85.1		7.3	
	3		8.4		0.7		85.5		7.1	
	Av.		8.1		0.8		85.2		7.2	
Average error			0.4		0.3		0.7		0.1	
Maximum error			0.9		0.7		1.7		0.2	



15 minutes. The condensers are rinsed with water and the solutions are cooled below 20° C. and titrated with approximately *N* sodium hydroxide. During this titration, the solution should be shaken vigorously and care should be taken not to overstep the end point. As an additional precaution against saponification of ethyl acetate during titration, it is well to add the alkali slowly.

#### DETERMINATION OF WATER

Regardless of the amount of water present in a solution, its water content may best be determined by the use of Karl Fischer reagent (1, 6). In applying this method, samples containing about 150 mg. of water should be measured from a Smith weighing buret and placed directly in freshly dried 150-ml. balloon flasks containing 25 ml. of anhydrous methanol. It is preferable to make the titrations electrometrically, using a dead-stop end point (8).

#### ANALYSIS OF SYNTHETIC MIXTURES

The results of a number of analyses of three synthetic mixtures, shown in Table II, are considered sufficiently accurate for a routine control method.

#### ACKNOWLEDGMENT

The author wishes to acknowledge the assistance of the late Walter Gunther in making many of the analyses.

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## Determination of Carbon-Linked Methyl Groups

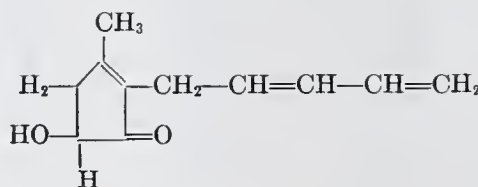
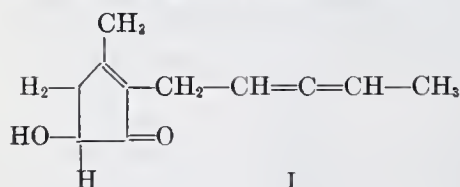
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IN CONNECTION with investigations of pyrethrolone (4) the authors have drawn important conclusions from the carbon-linked methyl content of various fractions and derivatives. For the determination of this grouping they have employed the method described by Pregl (5), which is essentially that of Kuhn and L'Orsa (3), based on chromic acid oxidation of the sample and titration of the resulting acetic acid. Since many determinations were required, some modifications and simplifications in the original method were introduced.

In general, little use has been made of terminal-methyl determinations in analytical studies of organic compounds, perhaps because theoretical values are seldom obtained except with certain types of groupings. Although in general straight-chain compounds furnish the theoretical yield of acetic acid with great precision, other groupings, such as a single methyl group attached to an aliphatic ring, usually yield somewhat less than 100%, and the result must be considered in connection with that furnished by a reference compound. In many cases where more than one methyl group on the same carbon atom is involved, or where methyl groups are attached to aromatic rings, this method seems to be of doubtful value.

The special problem was to distinguish between the two formulas for pyrethrolone—formula I (or similar compounds with the grouping C=CH—CH<sub>3</sub>) and formula II—and to estimate the proportions of each in mixtures.



The terminal-methyl determination of Pregl for carbon-linked methyl groups has been modified for more rapid determinations. The terminal-methyl number, or the number of mole equivalents of acetic acid produced from a mole equivalent of substance, has been determined for certain reference compounds.

In both formulas the methyl group attached to the pentenone ring would, from analogy with similar known structures, furnish about 0.8 mole of acetic acid per mole of compound, which is about the value that should correspond to formula II. Formula I should furnish a value of about 1.8, because of the second terminal methyl. In the special case of pyrethrolone and its derivatives very sharp and reproducible results were obtained as is shown in Table I.

In addition to making changes in the method, the authors have determined the amount of acetic acid furnished by a number of structures, especially of cyclic compounds.

#### EXPERIMENTAL

The changes made in the original directions consist in employing the apparatus (Figure 1) designed by Clark (1) for use in acetyl semimicrodeterminations, and in eliminating the reduction of the excess chromic acid with hydrazine.

From 20 to 30 mg. of sample are weighed on a piece of cigarette paper in the case of solids or, if a liquid, in a small glass capsule. The sample is placed in the oxidation flask, A, together with 5 ml. of cold oxidizing mixture made by adding 20 ml. of concentrated sulfuric acid to 16.8 grams of chromic anhydride dissolved in 100 ml. of water. The finger condenser, B, is put in the neck of the flask, and the mixture is refluxed over a microflame for 1 hour.

The finger condenser is then removed and washed free of acid with as little water as possible, the washings being allowed to run into the flask. Seven grams of magnesium sulfate are added and the flask is set up for steam distillation. The flame is replaced under the flask during the distillation in order to concentrate the contents of the flask while 50 ml. of distillate are being collected. The distillation is then titrated with a 0.05 *N* barium hydroxide solution to the neutral point of phenolphthalein.



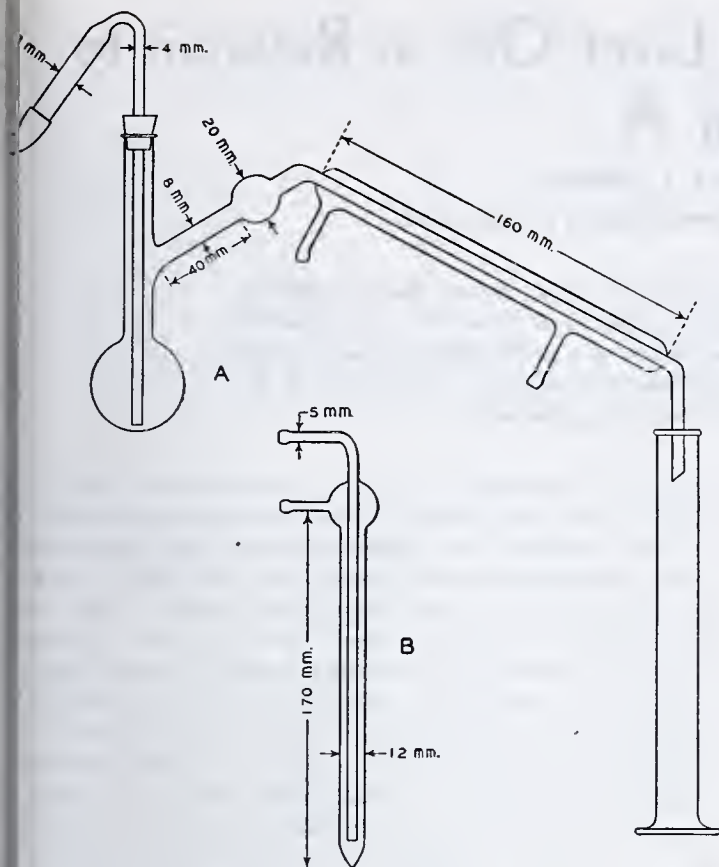


Figure 1. Diagram of Apparatus

An additional 5 ml. of distillate should not change the end point appreciably. A correction, determined by a blank experiment in which the organic material is omitted, is applied. Barium hydroxide is most convenient for titration, because any trace of sulfuric acid that might have been carried over is at once noticed. If sulfuric acid comes over, the determination must be repeated.

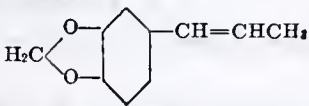
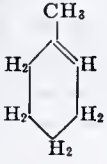
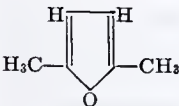
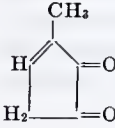
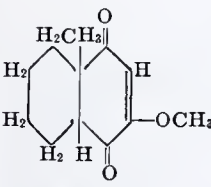
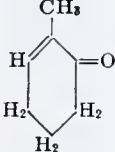
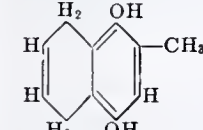
However, the Elek and Harte (2) iodometric procedure may be used at this point with equally good results. An advantage of the latter would be in correcting for any sulfur dioxide that might be carried over. In their work the authors have always used ample oxidizing mixture and have never encountered trouble with sulfur dioxide except when the solution was concentrated to the point where charring occurred during distillation. This can be avoided by not permitting the solution to become thick. Should there be a tendency for the solution to thicken, the flame is removed and passage of steam continued until the solution in the flask is once more fluid.

The terminal-methyl number is the number of mole equivalents of acetic acid produced from one mole equivalent of the

Table I. Precision of Method

Substance	Weight of Sample Gram	0.05 N Barium Hydroxide Ml.	Terminal-Methyl Number
Ethyl methyl ketone semicarbazone	0.0194	5.12	1.67
	0.0193	5.07	1.66
Citonic acid	0.0190	4.25	0.94
	0.0174	3.96	0.95
	0.0226	5.19	0.95
	0.0266	6.16	0.98
Methylcyclohexene-2	0.0180	2.09	0.53
	0.0216	2.50	0.53
Pyrethron semicarbazone	0.0205	1.95	0.99
Fraction	0.0203	1.90	0.97
Pyrethrolone methyl ether	0.0218	3.07	1.30
Fraction	0.0216	3.08	1.32
Isobutylpyrethrolone	0.0202	3.20	1.83
Semicarbazone	0.0203	3.15	1.80
Pyrethrolone (fraction a)	0.0208	3.43	1.42
	0.0221	3.67	1.43
Pyrethrolone (fraction b)	0.0213	2.83	1.14
	0.0253	3.23	1.10
Sodium acetate (fused)	0.0235	5.72	0.98
	0.0183	4.80	1.05
	0.0260	6.37	0.99

Table II. Terminal-Methyl Numbers of Reference Compounds

Compound	Present Method	Found by Kuhn and L'Orsa by Acetic Acid Titration <sup>a</sup>
$\text{CH}_3\text{COONa}$ $\text{CH}_3\text{CH}=\text{CHCOOH}$	1.00 0.96	1.00 0.85
	0.76	0.85
	0.53	....
	1.70	....
$\text{C}_2\text{H}_5(\text{CH}_3)\text{C}=\text{NNHCONH}_2$	1.66	1.70
	1.00	....
	0.29	....
	0.80	0.80
	1.00	....

<sup>a</sup> Kuhn and L'Orsa results were given for general structures.

Last four compounds obtained through courtesy of L. W. Butz, Bureau of Animal Industry.

compound. The calculations are made according to the following formula:

Terminal-methyl No. =

$$\frac{\text{normality of alkali} \times (\text{ml. of titer} - \text{blank}) \times \text{mol. wt. of sample}}{\text{gram of sample} \times 1000}$$

EXAMPLE. 0.0194 gram of ethyl methyl ketone semicarbazone (mol. wt. 129.16) required 5.12 ml. of 0.05 N barium hydroxide. The blank was found to be 0.10 ml.

$$\frac{0.05 \times (5.12 - 0.10) \times 129.16}{0.0194 \times 1000} = 1.66$$

Table I illustrates the precision of the method. Table II contains the results from various typical compounds, which may serve for reference.

The results may also be expressed in terms of per cent  $\text{CH}_3$ .

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# Spectroscopic Study of Fish Liver Oils in Relation to Vitamin A

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Eight fish liver oils and three samples of U.S.P. reference oil were studied spectroscopically and conversion factors calculated from the biological potencies of these oils. Characteristic curves were studied and it was emphasized that measurement at a single ultraviolet wave length is insufficient for a proper spectroscopic evaluation of vitamin A content. The stability of the reference oils was studied at intervals during 6 months.

**D**URING the course of investigations concerning the use of direct ultraviolet spectrophotometry for the determination of vitamin A in certain agricultural products, a group of fish liver oils with a wide range of potencies and several samples of U.S.P. reference oil were studied. Characteristic absorption curves were obtained and comparisons were made between the spectroscopic values observed and those obtained by the company which supplied the oils. Biological potencies were also made available by the company.

## EXPERIMENTAL PROCEDURE

Most samples were studied without saponification, the oil being simply dissolved in purified hexane and examined spectroscopically over the region from 3100 to 3800 Å. The photoelectric spectrophotometer was the same instrument and was used under the same conditions as reported earlier (19) in work on crystalline vitamin A. Some samples were saponified in a manner similar to the procedure used for butterfat (21) and the final solutions in ether were studied spectroscopically. The commercial fish liver oils were stored at  $-20^{\circ}\text{C}$ . Spectroscopic examination of the extracts or solutions was made immediately after preparation.

U.S.P. reference oils were studied when received and after periods of storage in the original opened bottle. Sample I was stored at  $-20^{\circ}\text{C}$ . and samples 2 and 3 at  $+5^{\circ}\text{C}$ .

## RESULTS AND DISCUSSION

Table I presents data on the commercial oils and the corresponding conversion factors calculated for the conversions of spectroscopic to biological units. The mean conversion factors agreed within 3%. For four samples, the values obtained were identical with those reported by the company. Differences found in several other samples probably indicate that changes occurred in the time between the two absorption measurements.

Table II contains data observed on the reference oils. Results obtained by different operators or on duplicate samples were reproducible to approximately  $\pm 1\%$ . The three samples were different when first received, with differences in absorption of

$\pm 8\%$ . Determination of the conversion factors, made after storage of the opened bottles, showed a maximum deviation from the mean similar to that of the fresh samples, although average values of the opened samples agreed within  $\pm 0.5\%$  with the averages of values for the corresponding fresh samples. The respective averages from fresh and opened bottles agreed equally well whether studied before or after saponification. The average conversion factor for the U.S.P. reference oils (whole) is considerably lower than the corresponding value for the commercial oils studied. As expected from the nature of the biological determination, the maximum deviation of the conversion factors for the commercial oils are higher than those for the reference oils. For 3-month periods, the stability appeared good at these temperatures. Between 3 and 6 months, however, sample 1 increased considerably in absorption as found also by Oser, Melnick, and Pader (15). Such oils have often been reported to lose absorption in the ultraviolet during storage (3, 4, 12). The time involved is probably important.

In Figure 1 are representative characteristic curves of the oils (both whole and the unsaponifiable fraction) with a wide range of potencies. These are compared with corresponding curves of crystalline vitamin A (19) and the unsaponifiable fraction of a reference oil. All curves are arbitrarily placed to coincide at 3800 Å., so that differences in shape are most evident in the region of maximum absorption. If they had been placed to coincide at 3240 Å., agreement would have been excellent down to 3100 Å. but the curves would have diverged from 3240 to 3800 Å. in the reverse order from the divergence shown in Figure 1. Extraneous absorption is thus plainly evident in all the oils. The curves for the unsaponifiable fractions approach that of vitamin A more closely than the curves of the corresponding parent oils. Curiously, the curves for the reference oil and the 2300 I.U. (No. 62331) lie between those for the two oils of much higher potencies.

**COMPARISON WITH OTHER WORK.** There are comparative few papers in the recent literature of this subject which present full absorption curves or data for oils or the unsaponifiable fractions. From examples given here and elsewhere (3, 4, 7, 12, 14, 15), it becomes increasingly evident that more attention should be paid to the characteristics of the curve as a whole (in comparison with known vitamin A-potent substances) in the interpretation of the absorption value obtained at the position of the maximum for vitamin A, which is the point usually used for such determinations.

The importance of characteristic curves as approximate indexes of relative reliability for vitamin analysis should always be kept in mind. Knowledge of the source, treatment, and conditions of storage of oils is always an aid in the interpretation of ultraviolet spectroscopic observations.

Such problems have been discussed by Wilkie (13) and Morton (14). Recent work on the spectroscopic

Table I. Spectroscopic and Biological Data on Fish Liver Oils

Sample	Biological Potency (Company Value) U.S.P. u./g.	Spectroscopic Data $E_{1\text{cm.}}^{1\%}$ (3240 Å.)			Conversion Factor $E_{1\text{cm.}}^{1\%}$ (3240 Å.)	
		Company value (hexane) <sup>a</sup>	Whole (hexane)	Unsaponifiable fraction (ether)	Company value (hexane) <sup>a</sup>	Whole (hexane)
62331 (Cod)	2,300	1.18	1.19	1.18	1950	1930
58581 (Cod)	2,500	1.06	1.01	1.00	2360	2490
60771 (Halibut)	62,000	27.8	29.7	28.5	2230	2090
30539 (Halibut)	62,000	30.1	30.1	...	2060	2060
30579 (Mixture)	75,000	37.1	43.2	...	2020	1740
31469 (Halibut)	84,000	40.5	44.0	...	2070	1910
62861 (Halibut)	157,000	74.7	74.8	76.0	2100	2100
33019 (Mixture)	445,000	202	203	...	2200	2190
		Mean			2124	2064
		Mean absolute deviation, %			4.9	7.45
		Maximum deviation, %			11.1	20.6

<sup>a</sup> Whole in hexane.



recovery of vitamin A in oils from different sources (9) presents further difficulties in application to miscellaneous products. The problem of multiplicity of vitamin A-potent substances, suggested by Coy, Sassaman, and Black (3) to explain differences between conversion factors for cod liver oil compared to richer oils, is receiving considerable attention at present. The possible presence of vitamin A<sub>2</sub> (10) and anhydro vitamin A (16) makes a knowledge of the source important. For certain types of materials, subvitamin A (5) and kitol (6) may complicate the spectroscopic picture.

McFarlan, Bates, and Merrill (12) concluded that the shapes of absorption curves for distilled vitamin A esters and the unsaponifiable fraction of fresh U.S.P. reference oil are identical. Since their work was reported, vitamin A has been more highly purified and spectra of such preparations do not show the shelf on the long wave-length side of the maximum (19). The characteristic curves of the authors' samples of reference oil (unsaponifiable fraction) were not identical with their curves for vitamin A. This agrees with the work recently reported by Oser, Melnick, and Pader (15).

The conversion factors for reference oils after saponification agreed well with the mean value of 2152 found by Ewing, Vandembelt, Emmett, and Bird (8) for a group of commercial oils.

The average of the conversion factors from sections 1 and 2 of Wilkie's 1941 report (18) on the first U.S.P. reference oil is 2060, which agrees with values reported here on the saponified reference oils.

The differences between fresh and opened bottles are less than those reported by Coy, Sassaman, and Black (3) for full and partially filled bottles of reference oil and the conversion factors are lower than for their oils. These differences might reasonably arise from the fact that they used a more potent oil (3000 U.S.P.) although their recent comparison (4) of such reference oils of different potencies is contradictory to this. Average deviations reported by them for many other oils are comparable to the authors', although their factors are higher in value.

Little (11) reported a factor of 2240 calculated from direct spectrophotometry of the unsaponifiable fraction of a reference oil (1700 U.S.P.) in cyclohexane. By irradiation methods he raised this value to 2540, which is very close to the figure calculated below for pure vitamin A in hexane, a similar solvent.

A very extensive comparison of physical methods for the determination of vitamin A, as employed in many laboratories, as reported by the Subcommittee on Physical Tests of the American Pharmaceutical Association (1). The corrected conversion factors from selected laboratories which produced the most concordant results (Table IX) ranged from 1883 to 2283, with an average value of 2064 (isopropanol), which agrees well with the mean values obtained on the commercial oils before saponification and on the reference oils after saponification (measured in hexane and ether, respectively). Solvent differences could not be overlooked in such comparisons (13, 19).

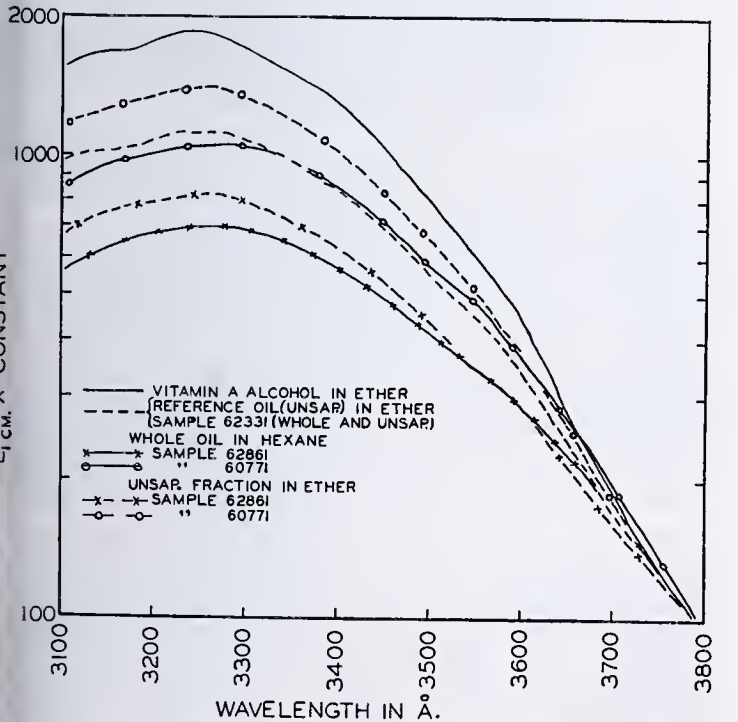


Figure 1. Ultraviolet Absorption Spectra of Fish Liver Oils  
Constant used in ordinate scale is adjusted for each curve to cause all curves to coincide at 3800Å with that of vitamin A alcohol.

Table II. Spectroscopic Study of Variation and Stability of Reference Oils

Sample	Rated Biological Potency <i>U.S.P.</i> <i>u./g.</i>	Date	Spectroscopic Data $E_{1\text{cm.}}^{1\%}$ (3240 Å.)		Conversion Factors $E_{1\text{cm.}}^{1\%}$ (3240 Å.)	
			Whole (hexane)	Unsaponi- fiable fraction (ether)	Whole (hexane) <i>U.S.P.</i> <i>u./g.</i>	Unsaponi- fiable fraction (ether) <i>U.S.P.</i> <i>u./g.</i>
1	1700	4-7-42	0.890	0.758	1910	2240
		5-22-42	0.897	...	1895	...
		7-14-42	0.904	0.750	1880	2270
		10-22-42	...	0.867	...	1960
2	1700	7-14-42	0.934	0.824	1820	2060
		8-10-42	...	0.829	...	2050
		10-22-42	...	0.822	...	2070
3	1700	10-22-42	...	0.886	...	1920
		Average of fresh bottles			1865	2073
		Maximum deviation, %			...	8.0
		Average of opened bottles			1887	2088
		Maximum deviation, %			...	8.7

GENERAL CONSIDERATIONS. The deviations of the authors' factors for these oils were of the same magnitude as in attempted similar correlations between spectroscopic and biological values in the case of the unsaponifiable fractions of certain butterfats (20). The much higher potencies of these oils might be expected to permit smaller deviations than were obtained from the butterfats, which contained comparatively a much lower concentration of vitamin A.

Close correlation with biological results is difficult to attain if a uniform constant or conversion factor is considered, especially for different types of material. Morgareidge (13) has pointed out the usefulness of a factor for comparative purposes only, without any assumption that the result is the true biological potency. He and Oser, Melnick, and Pader (15) have noted that the reference oil is a poor basic material for the establishment of a spectroscopic standard.

From the biological potency of vitamin A reported by Baxter and Robeson (2), factors of 2570 and 2350 may be calculated for pure vitamin A in hexane (19) and ether (21), respectively. The difference between these factors and the corresponding values found for oils indicates the magnitude of the errors caused by extraneous absorption and other uncertainties, such as biological availability. Variations in biological availability of vitamin A-potent substances may be expected always to limit the minimum size of the deviations in such correlations of spectroscopic with biological values.

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# Determining Chlorophyll, Carotene, and Xanthophyll in Plants

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A method for the determination of chlorophylls a and b, carotene, and total xanthophyll from a single ether solution is described. This method is adaptable to any spectrophotometer of good resolving power provided preparations are available for determination of absorption constants for the instrument to be used. The pigments are extracted from the plant material with acetone, transferred to ether, and chlorophyll is determined from the light absorption at the wave lengths of the chlorophyll a and b maxima in the red end of the spectrum. Carotene and xanthophyll are separated in the unsaponified ether solution by means of the flowing chromatographic technique and then determined spectrophotometrically. Evidence is presented of the reproducibility of results which may be expected with this method. Single determinations seldom vary more than 5% from the average of four similar samples in the case of each pigment determined.

**A** RAPID, accurate method which requires limited laboratory equipment for the determination of chlorophyll, carotene, and xanthophyll in plant materials is desirable when these constituents must be determined in a large number of samples. Previous methods require at least two separate extracts and a rather long procedure for the determination of carotene and xanthophyll. The chlorophyll a and b, carotene, and xanthophyll contents of an ether solution of a plant extract may be determined by the method described in this paper. Chlorophyll is determined by making light-absorption measurements on dilutions of the solution. Carotene and xanthophyll are separated on an adsorption column, using the flowing chromatographic technique.

In the usual methods for carotene and xanthophyll determination, chlorophyll is removed by saponification and the xanthophyll is separated from the carotene by partition between methyl alcohol and petroleum ether. Miller (8) and Wall and Kelley (12) found that such a partition was not complete, since some noncarotene pigments remain in the petroleum ether layer.

Curtis (4), Moore (9), Wall and Kelley (12), Fraps and Kemmerer (5), and others have used the chromatographic technique as a means of separating the carotene fraction from other pigments in plant extracts. All these workers used petroleum ether as the solvent and various adsorbents, including dicalcium phosphate, soluble starch, and adsorptive magnesia-Celite mixtures. Adsorptive magnesia-Celite mixtures have been used extensively by Strain (11) in separating carotene and leaf xanthophylls and also by Zscheile, White, Beadle, and Roach (16) and other workers. Bode (1) used a petroleum ether solution of the pigments and a powdered sugar column, and obtained carotene in the solution which passed through the column, sectioned the column into chlorophyll b, chlorophyll a, and xanthophyll zones, eluted them separately, and measured the pigments against standards in a colorimeter. Though his method is neither quick nor very accurate, it appears to be the only previous method involving the analytical separation of both carotene and xanthophyll in unsaponified plant extracts.

## ABSORPTION SPECTRA MEASUREMENTS

**ABSORPTION METHODS.** A Cenco-Sheard Spectrophotometer described by Sheard and States (10) was used in this work. The light source was an 18-ampere 6-volt incandescent light bulb. A 5-cm. carriage permitted the use of 5-, 2-, and 1-cm. solution cells. To eliminate the possibility of stray light from the second-order spectrum, a red filter which removed all radiation below 600 mμ was used in making all readings above 640 mμ. It was found that log  $I_0/I$  values obtained between 600 and 640 mμ were the same with or without the filter. A 1.5-mm. entrance slit and a 2.5-mμ exit slit were used in making chlorophyll determinations. For carotene and xanthophyll determination an entrance slit of 2.0 mm. was necessary to obtain sufficient light intensity for accurate work when the 2.5-mμ exit slit was used. According to Sheard and States (10) the total region isolated in the chlorophyll determinations was 8.5 mμ and in the carotene and xanthophyll determinations 10.5 mμ, with most of the radiant energy within a 2.5-mμ region. The Beer-Lambert law was used in the form presented by Gibb (6). This is identical with the form used by Zscheile and Comar (15) except for the symbols used. The equation is:

$$\log_{10} \frac{I_0}{I} = Kl = kcl$$

where

- $I_0$  = intensity of radiant energy transmitted by solvent-filled cell
- $I$  = intensity of radiant energy transmitted by solution-filled cell
- $K$  = extinction coefficient
- $l$  = thickness of solution
- $k$  = specific extinction, a constant at any particular wavelength for a pure pigment obeying the Beer-Lambert law
- $c$  = concentration in grams per liter

For maximum accuracy the concentrations and cell length were adjusted to keep the log  $\frac{I_0}{I}$  value within the range 0.200 and 0.800 as recommended by Zscheile and Comar.

**ABSORPTION CONSTANTS.** *Chlorophyll.* Zscheile and Comar (15) determined the absorption constants of highly purified chlorophylls a and b and Comar and Zscheile (3) used these constants in determining the chlorophyll a and b content of plant extracts. An instrument as accurate as the one they used is not available in most laboratories and their method, therefore, cannot be applied without modification. Comar (2) determined the chlorophyll content of plant extracts by means of a Cenco-Sheard Spectrophotometer with a larger light source than was used in this work. By adjusting the instrument to read 660 mμ at the red absorption maximum of chlorophyll a, adjusting the concentration of the solution to give log  $\frac{I_0}{I}$  values of 0.5 to 0.8 at 660 mμ, and using the same solution for the reading at 642.5 mμ he was able to obtain results that agreed satisfactorily with those obtained on the more accurate instrument used by Comar and Zscheile. In this study the constants of Comar and Zscheile could not be used successfully with the same kind of instrument used by Comar. This may have been due to the use of a weaker light source and a wider entrance slit. By purifying the chlorophyll components and obtaining constants for the instrument used, satisfactory results were possible without adjusting the concentration to the narrow limits Comar found necessary.



Table I. Constants

Wave Length, mμ	Specific Extinction in Chlorophyll a	Ether Solution Chlorophyll b
599.5	9.95	9.95
642.5	14.2	57.5
661	97.0	5.0

The constants at various wave lengths for this instrument and preparations used are given in Table I.

The chlorophyll a maximum reported by most workers at 660 mμ was obtained consistently at 661 mμ on this instrument when it was calibrated at the 546 mμ line of mercury. Sheard and States (10) claim an accuracy of ±1 mμ at all wave lengths when the instrument is calibrated at this wave length and it seems probable that the different position of the maximum was an instrumental error. One of the crossing points of the chlorophyll a and b curves was obtained at 599.5 mμ on this instrument. The specific extinction at this wave length was used to calculate the total chlorophyll concentration as a check on the concentration calculated at the chlorophyll maxima.

Comar and Zscheile (3) have described in detail the determination by simultaneous equation of the concentration of each pigment in a binary system. Using their method, the following equations were derived from the constants in Table I and were used in determining the chlorophyll content of the authors' extracts.

Chlorophyll a = 10.44 (B<sub>661</sub>) - 0.91 (B<sub>642.5</sub>)  
Chlorophyll b = 17.61 (B<sub>642.5</sub>) - 2.58 (B<sub>661</sub>)

B<sub>661</sub> and B<sub>642.5</sub> are equal to the  $\frac{\log_{10} I_0/I}{l}$  values at 661 and 642.5 mμ, respectively, and the concentrations of chlorophylls a and b are expressed in milligrams per liter in the measured solution.

The method used in purifying the chlorophylls was based upon that of Zscheile and Comar (15). The details of the method used and the absorption curves of the chlorophyll preparations obtained will be reported later.

**Carotene.** A crystalline β-carotene sample was obtained through the courtesy of F. P. Zscheile. He states (14) that the sample analyzed 94.5 ± 1% β-carotene, that it contained approximately 1% colored impurities, and that the absorption curve of this sample was very close to the standard curve of his laboratory. The principal maximum in ether solution was found at 450 mμ and in 4% ether-96% petroleum ether at 448 mμ in this laboratory. The average values of the specific extinction at the maximum were 240.7 and 239.1, respectively. A mixture of ether and petroleum ether of intermediate composition was used in the analytical work. The maximum of the analytical solutions occurred at 449 mμ and 240 was used as the specific extinction value for determining the concentration of the analytical samples.

No evidence of α- or neo-β-carotene was obtained chromatographically or spectrophotometrically in fresh ether extracts of tobacco; consequently, it seems probable that the carotene was all or nearly all β-carotene. When the carotene fraction was allowed to stand for several days in the refrigerator, the absorption values at the secondary maximum fell in comparison with those at the primary maximum, indicating the production of neo-β-carotene. This was more noticeable in pure petroleum ether solutions than in a mixed solvent or in pure ether. Further studies of the carotene fraction extracted by this method are in progress.

ANALYTICAL METHODS

**METHOD OF EXTRACTION.** The ether extract was obtained by a modification of the method of Comar and Zscheile (3). Ten to 15 grams of leaf tissue, a small amount of calcium carbonate, and 125 to 150 ml. of acetone were placed in the cup of a Waring Blendor. A glass baffle plate, cut to fit the cross section of the Blendor container, was suspended about one third of the way down from the top. This prevented splashing of the sample into the cover, from which complete recovery was difficult. The sides of the container were washed down with acetone once during extraction to ensure complete extraction. After 5 minutes in the Blendor, the mascerate was filtered through paper in a Büchner

funnel and the residue was washed thoroughly with pure acetone. The volume of the filtrate was measured and a 100-ml. portion was added to 50 ml. of ether in a 250-ml. separatory funnel. One hundred milliliters of water were added and the water-acetone layer was removed. If xanthophyll determinations were to be made, it was necessary to extract the acetone-water layer once or twice more with 10 to 15 ml. of ether each time to recover xanthophyll which remained in this layer. No evidence of chlorophyll or carotene was observed when these extracts were examined chromatographically.

Acetone was removed from the combined ether extracts by washing with distilled water, using a modified form of the washing technique described by LeRosen (7). Instead of using a constant wash, water was allowed to fall dropwise from the fine-drawn tip of a glass tube above the surface of the ether, drawing the water layer off from time to time as it accumulated. Using this method, no tendency for chlorophyll loss from emulsion formation was experienced, although such a tendency was evident when the ether layer was scrubbed through distilled water by the method of Comar and Zscheile (3). Since they reported no such loss, it may be that this trouble was caused by some substance characteristic of tobacco extracts. Approximately 300 ml. of water were used in washing.

The ether solution was dried by placing the separatory funnel in a refrigerator maintained at 7° C. This low temperature decreased the solubility of water in the ether and the water layer was drawn off. The cooled ether was then transferred to an Erlenmeyer flask and anhydrous sodium sulfate was added to complete the drying. After at least an hour in the refrigerator, the ether solution was filtered through cotton to remove the sodium sulfate, the filter was washed, and the solution was made up to volume in a volumetric flask, usually 50 ml., at the temperature of the refrigerator.

**CHLOROPHYLL DETERMINATION.** In determining chlorophyll concentration 30 to 40 ml. of ether were placed in a 50-ml. volumetric flask, the stock ether solution was removed from the refrigerator, and by use of a Mohr pipet enough of this solution was added to the volumetric flask to give a chlorophyll content of 4 to 10 mg. per liter. With practice it was possible to estimate this concentration visually. The stock solution was returned to the refrigerator as soon as possible and the more dilute solution was made up to volume at room temperature. After thorough mixing, the 5-, 2-, and 1-cm. absorption cells were filled with this solution. The 5-cm. cells were placed in the instrument and a reading was made at 599.5 mμ, the 2-cm. cells were used in obtaining the reading at 642.5 mμ, and 1-cm. cells at the chlorophyll a maximum, which fell at 661 mμ on this instrument. By using the three lengths of cells, only one dilution was necessary to give log I<sub>0</sub>/I values between 0.2 and 0.8 for the three readings.

**CAROTENE DETERMINATION.** The carotene fraction was separated from the other pigments by passing a portion of the stock ether solution through an adsorption column containing a mixture of 5 parts by weight of magnesium oxide (Micron Brand No. 2641, Westvaco Chlorine Products Company, Newark, Calif.) and 3 parts of Hyflo-Super-Cel (Johns-Manville, New York). Carotene was unadsorbed, while the other pigments were retained on the column. A battery of three adsorption columns was used, all of which were connected to a constant-pressure air supply of about 5 cm. of mercury pressure. The adsorption columns were made in glass tubes about 15 cm. long with an outside diameter of 2.5 cm. The bottom of each tube was tapered to a point in which there was a small opening. A small piece of cotton tamped into the constricted portion of the tube retained the adsorbent. In forming the column a small amount of the adsorbent mixture was placed in the tube and 30 ml. of petroleum ether were added. More adsorbent was then added to make a total of 6 grams and any remaining on the sides of the tube was pushed into the petroleum ether by the use of a cork on the end of a glass rod. After standing for about 15 minutes, air adsorbed on the particles of adsorbent was stirred out of the column by gently moving a glass rod about in the adsorbent-petroleum ether suspension. The air supply was connected and the petroleum ether was forced through until 1 cm. of solvent remained above the adsorbent. Five milliliters of the stock ether solution of the plant pigments were added, and the ether-petroleum ether solution was run into the adsorbent. Twenty milliliters of pure ether were added to wash the carotene into a 25-ml. volumetric flask placed below the tube.

Shortly after the ether was added, the solvent dropping from the tip of the tube contained carotene. Carotene collected on the tip of the tube because of solvent evaporation and it was necessary to wash this into the flask at the end of the separation. The carotene fraction was completely removed from the column when 15 to 20 ml. of the solution had collected in the flask. If the solution dropping from the tube at this time was colored, xanthophyll was coming through and the separation had to be repeated.



Table II. Content of Carotene, Xanthophyll, and Chlorophylls a and b and Per Cent Chlorophyll a

(Calculated on oven-dry weight and area bases in leaves of tobacco plants grown in the greenhouse with and without artificial light)

Sample	Treatment	Chlorophyll											
		a	b	Total	a	b	Total	a	Carotene		Total Xanthophyll		
		Mg./g.	Mg./g.	Mg./g.	Mg./sq. m.	Mg./sq. m.	Mg./sq. m.	%	Mg./g.	Mg./sq. m.	K/g.	K/sq. m.	
1	Lighted	9.04	3.27	12.31	135.6	49.0	184.6	73.4	0.59	8.9	0.340	5.10	
2	Lighted	10.29	4.19	14.48	136.8	55.8	192.6	71.1	0.69	9.1	0.376	5.00	
3	Lighted	10.48	3.68	14.16	136.3	47.8	184.1	74.0	0.67	8.7	0.339	5.24	
4	Lighted	9.43	3.49	12.92	134.4	49.8	184.2	73.0	0.64	9.2	0.326	4.66	
	Av.	9.81	3.66	13.47	135.8	50.6	186.4	72.9	0.65	9.0	0.345	5.00	
5	Unlighted	12.74	4.74	17.48	177.9	66.2	244.1	72.9	0.82	11.4	0.482	6.72	
6	Unlighted	11.98	4.63	16.61	165.0	63.8	228.7	72.1	0.80	11.0	0.456	6.30	
7	Unlighted	12.20	4.43	16.63	161.5	58.7	220.2	73.3	0.84	11.2	0.456	5.80	
8	Unlighted	12.62	5.08	17.70	162.1	65.3	227.4	71.3	0.88	11.3	0.490	6.40	
	Av.	12.39	4.72	17.11	166.6	63.5	230.1	72.1	0.84	11.2	0.471	6.31	

The carotene solution was made to volume with diethyl ether at room temperature and light-absorption measurements were made at 449  $\mu$ .

The petroleum ether used in forming the column slowed down the movement of the xanthophylls and unless there was an uneven movement of the xanthophyll bands no difficulty was experienced in the separation. Although the use of a mixed solvent is usually undesirable, because of difficulties involved in maintaining a given concentration of the solvent, no trouble was encountered in this work. This was undoubtedly due to the close agreement in absorption value in ether and in petroleum ether solutions discussed above. There was no change in analytical results when diethyl ether instead of petroleum ether was used in forming the column. The use of diethyl ether required more absorbent, and more time for the separation. When carotene fractions were passed through a second column, 95 to 100% recovery of the pigment was obtained.

**XANTHOPHYLL DETERMINATION.** Xanthophyll was determined on the same column used to obtain the carotene fraction. To obtain the total xanthophyll fraction, anhydrous ethanol or an ethanol-ether mixture was added to the column after the carotene had been removed. This caused elution of the xanthophylls which were collected in a volumetric flask in the same way as the carotene fraction. The amount of ethanol required depends upon the plant material analyzed and upon other variables. Too low concentrations of ethanol do not elute all the xanthophyll, whereas too high concentrations elute some green pigments. The xanthophyll solution contains both ether and ethanol. When the mixture remains fairly constant, comparable results can be obtained by analyzing the solution directly. For more accurate work the ethanol can be removed by washing with water.

If specific extinction values are known for the xanthophylls of the plant analyzed it is theoretically possible to calculate the concentration of the individual xanthophylls from a solution of the total xanthophyll components, using methods similar to those used for chlorophylls a and b. It is also possible in some instances to separate the different xanthophylls by gradually increasing the concentration of alcohol added to the column. In these instances the different xanthophylls may be collected successively by the flowing chromatographic technique or the column may be removed from the tube and the bands may be eluted individually. In the present work the absorption curve of the total xanthophyll fraction was determined and the samples were run at the wave length of maximum absorption, which was 442  $\mu$ . The extinction coefficient,  $K$ , was then calculated. If the proportion of the different xanthophylls remains constant,  $K$  is proportional to the concentration and therefore is satisfactory for comparative purposes.

#### REPRODUCIBILITY AND ADVANTAGES OF METHOD

Typical results of the use of this method are presented in Table II to show the degree of reproducibility obtainable.

Part of a crop of tobacco grown in the greenhouse in midwinter was supplied with natural light and part with natural light plus

supplementary light from a tungsten filament source in the morning and evening, to make a 16-hour total daily exposure. The intensity of the supplementary light was about 10 to 15 foot candles. Two-leaf samples were taken at corresponding height from each group of plants. The area of the leaves was determined by means of the equation of Young and Jeffrey (13) and one half of each leaf web was used for moisture determination and the other half for preparation of the ether solution. Two samples were taken from each group of plants and two measured portions were taken from each acetone solution. One portion of each solution (samples 1, 3, 5, and 7) were analyzed on the same day as taken and the other (samples 2, 4, 6, and 8) on the following day. The results are calculated on the oven-dry basis and also on the basis of leaf area.

The maximum deviation of any analysis from the average of the four similar analyses is less than 5% in most instances. In only three of the eighty cases does the deviation exceed 10% and these are all chlorophyll b determinations. Plants receiving artificial light to increase the length of day contained about 20% less chlorophyll, carotene, and xanthophyll than those not receiving supplementary light. Since these differences are large enough to be significant, further studies are being made to obtain more information on the effect of day length on the plant pigments.

The advantages of the method presented here are: (1) The equipment used is present in, or at least can be obtained by, the majority of modern laboratories. (2) Chlorophylls a and b, carotene, and total xanthophyll are determined from the same ether solution. (3) The sample is not subjected to high temperature nor to strong reagents which may cause changes in the pigments.

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# Preparation and Reclamation of Copper-Chromium Oxide Catalyst

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Carborundum aggregates, Celite pellets, and granular Celite have been tested as supporting materials for copper-chromium oxide catalyst and this combination has been found satisfactory for dehydrogenation of alcohols. The usual acetic acid leaching and washing of the catalyst may be omitted if adequate heat treatment at 150° C. is substituted. Copper-chromium oxide catalyst which has become sluggish in its action through prolonged use may be reactivated or reclaimed by heating to expel absorbed organic material and reoxidize the reduced metals. Acetic acid leaching and washing then provide a catalyst as active as the original for the dehydrogenation of alcohols.

yields but averages under normal and comparable operating conditions.

Experience has demonstrated that copper-chromium oxide deposited on granular Celite may be reactivated or reclaimed by carefully heating the used catalyst in an open container to expel all absorbed organic materials and reoxidize the reduced copper and chromium. This heating should be carefully controlled to prevent reduction of catalytic activity due to a change of the catalyst from an amorphous state to the crystalline structure (8). This heated catalyst was then leached with one 600-ml. portion of 10% acetic acid and further with six 600-ml. portions of distilled water. After drying at 125° for several hours the catalyst displayed greater activity than the original catalyst. Representative values for this reclaimed catalyst are also included in Table I.

Table I. Products Recovered by Dehydrogenation of 100 Grams of Butanol-1 with Six Copper-Chromium Oxide Catalysts

Products	Carborundum Aggregates		Celite Pellets		Granular Celite		Re-claimed Celite
	Catalyst A	Catalyst B	Catalyst A	Catalyst B	Catalyst A	Catalyst B	
Butanal, grams	59.8	57.0	58.7	56.1	52.0	57.8	60.0
Butanol-1, grams	3.7	3.8	4.1	5.3	1.7	5.0	2.0
Butyl butyrate, grams	2.4	4.6	2.2	2.8	6.1	1.5	4.3
Condensation products, grams	2.1	3.2	3.6	3.4	4.1	3.4	3.3
Gases collected, liters	28.0	27.5	27.4	26.2	27.2	27.2	27.7
Water, grams	3.0	3.6	4.5	4.9	4.5	4.9	3.9
Butene, by difference, liters	9.4	9.8	9.1	8.7	11.0	9.2	9.0
Grams	23.5	24.5	22.7	21.8	27.5	23.0	22.5
Hydrogen, from butanal, liters	18.6	17.7	18.3	17.5	16.2	18.0	18.7
Grams	1.7	1.6	1.6	1.6	1.5	1.6	1.7
Total products recovered, grams	96.2	98.3	97.4	95.9	97.4	97.2	97.7
Mechanical loss, grams	3.8	1.7	2.6	4.1	2.6	2.8	2.3

## SUMMARY

Two new inert carriers, Celite Carrier Type VII and Carborundum Brand porous aggregates, have been found satisfactory for supporting copper-chromium oxide in the dehydrogenation of alcohols.

A new method for the preparation of copper-chromium oxide catalyst, satisfactory for the dehydrogenation of alcohols, produces a catalyst of essentially the same initial activity, but this activity is lost more readily with prolonged use.

A method is described for the reactivation or reclaiming of used copper-chromium oxide catalyst when precipitated and decomposed upon inert carriers.

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COPPER-chromium oxide catalyst deposited upon pumice was first used for the dehydrogenation of alcohols by Dunbar, Cooper, and Cooper (6) in 1936, and extended to other inert carriers in 1938 (5). Additional porous and inert carriers have since been obtained and tested for like purposes. Several variations in the preparation or reclamation of copper-chromium oxide have been reported from time to time (1-4, 7, 8) and other variations have now been studied.

## EXPERIMENTAL PROCEDURE

One hundred-gram portions of butanol-1 were used to check the efficiency of the several catalysts because of the tendency of this alcohol to give consistent yields of aldehyde and a minimum yield of unsaturated hydrocarbon and ester. The equipment and procedure used in this study were the same as those previously described (5). Two methods were used in preparing the copper-chromium oxide catalyst, as well as a method for reclaiming the used catalyst.

The first method was identical with that previously described (5) except that two new types of inert carriers were substituted (catalyst A). The new supporting materials are Celite catalyst carrier Type VII, 6- to 10-mesh U. S. Standard screen size supplied by Johns-Manville, Manville, N. J.; and Carborundum Brand porous aggregates, Mix 70-120-G3, 10- to 20-mesh, supplied by the Carborundum Company, Niagara Falls, N. Y. This Celite Type VII is a porous variety of pellets with greater mechanical strength than the granular Celite which has previously been used.

In the second method of preparing the copper-chromium oxide catalyst (catalyst B) in place of the usual acetic acid leaching and washings with distilled water (5), the catalyst was heated in a hot-air oven at 150° C. for approximately 10 hours to expel ammonia, oxides of nitrogen, etc. A water extract of this catalyst was neutral to litmus. In the preparation of catalyst B the actual amount of copper-chromium oxide deposited upon the various inert carriers was increased by 15 to 25%, as compared to catalyst A. The granular Celite catalyst, as well as the reclaimed catalyst, was precipitated and decomposed upon Celite, Grade C-12,212.

Representative determinations for six catalysts on three different supporting materials are tabulated in Table I. All values included are the average of several determinations. The temperature of the catalyst was maintained at 300° to 325° C. The values for hydrogen are derived theoretically from the amount of aldehyde isolated, and the volume of butene is the difference between total gases collected and calculated hydrogen. This procedure is justified by representative analyses of such gaseous mixtures. The amount of water represents loss in weight of the distillate on drying with anhydrous sodium sulfate. All other values are actual amounts of material obtained by fractionation. The yields of butanal do not necessarily represent maximum



# Determination of the Dry Hiding of Pigmented Coatings

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An empirical equation expressing the relationship between the contrast ratio of a dry pigmented coating and the weight of material applied is developed by rectification of the dry hiding curve, using the method of averages (6). The equation is shown to apply to a series of four unrelated paints, and furnishes the means for calculating the dry hiding at any desired contrast ratio. The method presented yields reproducible results, provides the opportunity for neutralization of errors introduced by faulty technique, and will also function where high dry hiding pigments are used. The equation is applied successfully to data presented by other investigators where the range of contrast ratios covered reaches 0.999. A comparison between the derived equation and other empirical hiding power equations indicates excellent agreement in the case of white paints and a definite variation in the case of colored coatings. Theoretical and practical interpretations of the data and comparisons are discussed.

ONE of the controlling factors in the raw material cost of a pigmented coating is its opacity or hiding power, since in most cases the hiding pigment is the costliest portion of the paint when calculated on volume basis. It is therefore necessary to have an accurate means of determining the hiding power of a paint both for formulating purposes and for estimation of the quantity of material required to coat a given area. The methods for measuring hiding power in use at present are the brushout (8, 11, 12, 17, 20, 21) and cryptometer (10, 22, 23, 27, 28) for the wet hiding power and the dry film contrast ratio specification for the dry hiding power (7).

The A.S.T.M. method (1) for relative dry hiding has not received wide acceptance and does not appear in government specifications. A number of systems based on photometric or photographic measurement are described in the literature (2-5, 14, 15, 16, 25, 29). Stutz and Haslam (26) presented a general evaluation of the methods available at the time for hiding power determination, listing their individual shortcomings.

Mathematical relationships between paint film constants were derived by Kubelka and Munk (19) and Hanstock (13). The equation of Kubelka and Munk correlating the contrast ratio of a paint film over black and white against its reflectance at infinite film thickness was tested by Judd (18), who found that it did not hold closely in all cases. Hanstock indicates a relationship between the log of the quantity of light transmitted through a paint film and the thickness of the film. Sawyer (24) presents a comprehensive outline of the theoretical aspects of hiding power. His paper evaluates the applicability of the Kubelka and Munk equation, the equation derived by Bruce, and an empirical equation by R. H. Fell, who shows a linear relation between log 10 contrast ratio and reciprocal film thickness over the range of from 0.7 upwards nearly to 1.0 contrast ratio. The Fell equation has been checked by a number of workers and found to hold very closely in the case of white paints.

The disadvantages of the brushout method for hiding power determination are the difficulty of reproducing results by different operators, the large error introduced by faulty technique, and the fact that this method does not take into account loss of hiding of gloss paints on drying and the decided increase in hiding of flat paints formulated with high dry hiding pigments. The use of the cryptometer does not overcome these faults. The dry film contrast ratio method specifies a contrast ratio (less than one) for a given volume of paint per square foot, painted over a standard brushout sheet. Thus, results of this method are relative and cannot be used to calculate the quantity of paint needed to give complete covering. The ideal hiding power value would be that obtained over a standard surface when the contrast ratio is

such that the eye can no longer discern any difference in opacity over black and white. Bruce (4) assigned a value of 0.98 as the contrast ratio representing complete hiding, but Sawyer (24) quoted a reference to work by Kraemer and Schupp who showed that the critical contrast ratio is usually higher than 0.98 and may even be higher than 0.995 in some cases. The empirical equation presented by Bruce was reported as unsatisfactory by Gamble and Pfund (Sawyer, 24) and by the Baltimore Production Club (9). The Fell equation has been applied successfully to white paints but no data were found on its application to colored paints.

In order to eliminate the disadvantages of the methods and equations previously used in determining hiding power, work was undertaken in the authors' laboratory to find a means of measuring contrast ratio which could be duplicated with reasonable accuracy and to develop a general equation for the contrast ratio curve which would apply for white and colored paints and would hold in the higher range of contrast ratios.

## PREPARATION OF EXPERIMENTAL COATINGS

In order to determine whether any one equation would apply in all cases, a series of four unrelated paints was ground off. Pigments were selected which varied widely from each other in color and covering power. The extending pigments and vehicle were different in each case, as well as the gloss of the dry films. No attempt is made to correlate the results with any individual component of the paints, since the object of this investigation is to find an equation which can be applied regardless of the composition of the coating being tested. A formula breakdown of the test coatings is given in Table I.

## PREPARATION OF TEST PANELS

Six brushouts were made of each paint on standard checkerboard paintout sheets, which had an area of 1 square foot consisting of 17 white squares with a diffuse daylight reflectance of approximately 80% and 18 black squares with a reflectance of less than 5%. The brushouts were made as carefully as possible, the amount of paint being varied to obtain as wide a range of contrast ratios as possible. In the case of the strongly opaque green paint, it was necessary to thin the coatings in order to get the lower contrast ratio readings, while in the case of the more transparent Burgundy lake, two coats were used to get the higher readings. The test panels were permitted to dry for 24 hours.

## CONTRAST RATIO DETERMINATIONS

The photovolt reflectometer, used to measure the contrast ratio, is illustrated in Figure 1. This instrument consists of an indicating taut-wire suspension galvanometer, actuated by a search unit which is constructed so that readings are obtained

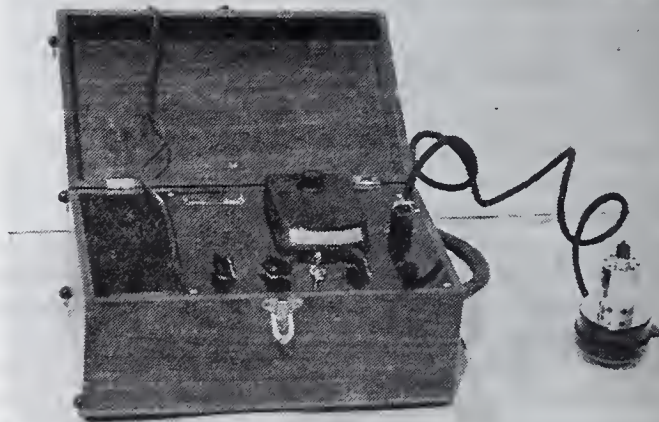


Figure 1. Apparatus for Measuring Contrast Ratio

<sup>1</sup> At present in the United States Navy.



Table I. Composition of Experimental Coatings

Coating	Finish	TS-W	TS-V	TP-V	HP-V	Wt./Gal. Pounds
		%	%	%	%	
1. White	Flat	83.5	62.0	78.0	30.2	15.1
2. Chrome green	Semigloss	83.3	66.0	55.5	7.5	13.3
3. Zinc chromate	Semigloss	68.3	62.5	42.0	66.5	11.0
4. Burgundy lake	Gloss	80.0	62.8	48.8	14.6	12.1

	Hiding Pigment	Extender	Vehicle
1. White	Titanium dioxide	CaCO <sub>3</sub>	Bodied linseed oil
2. Chrome green	Chromium oxide	Magnesium silicate	30 - gallon - dehydrated castor oil, maleic gum varnish
3. Zinc chromate	Zinc chromate	Diatomaceous silica	Alkyd resin, phenol resin, tung oil varnish
4. Burgundy lake	Burgundy lake	CaCO <sub>3</sub>	45-gallon ester gum, linseed oil varnish

TS-W, total solids by weight  
TS-V, total solids by volume  
TP-V, total pigment volume  
HP-V, hiding pigment volume  
Wt./Gal., weight per gallon in pounds

equivalent to a 45° angle of incidence and 0° viewing angle. The light source was a 15-cp. lamp, and a filter was used which made the light spectrally equal to average daylight. Reflectance readings were taken on the 15 squares in the middle of the test sheets, omitting the outer row. The readings over black and white were averaged separately and the averages divided to give the contrast ratio. A variation of more than 2% between the highest and lowest reading over each set of squares of the same color was considered cause for rejection and a new paintout was made. The contrast ratio data are given in Tables II to V.

RECTIFICATION OF CONTRAST RATIO CURVE

The procedure used to set up empirical equations and test their applicability to the experimental contrast ratio data was the method outlined by Davis (6). Briefly, it involves rectification of the curve by plotting those functions of the variables that produce a straight line. The proper functions are ascertained by trial and error.

In view of the work by Hanstock (13), the first relationship tried was that between the logarithmic functions of the weight and contrast ratio. However, plotting the data on log and semilog paper resulted in curves, which indicated that there was no proportional relationship between those functions. Since the contrast ratio curves are definitely hyperbolic, the next rectification attempted was the plotting of the reciprocal of the weights against the reciprocal of the contrast ratio. These functions would produce a straight line if the curve were an equilateral hyperbola. The resultant slight curve was an indica-

tion that additional constants were required and the following general equation for hyperbolic curves was investigated:

C = (W - W¹) / (a + bW) + C¹

where C = contrast ratio  
W = weight of paint to give contrast ratio of C  
C¹, W¹ = any set of coordinates on contrast ratio curve  
a, b = constants for contrast ratio equation

For this equation to hold, a plot of (W - W¹) / (C - C¹) against weight must yield a straight line. The derivation of the equation and proof of the rectification method are covered thoroughly by Davis (6). The function (W - W¹) / (C - C¹) was calculated for each paint and the data are presented in Tables II to IV. Plotting these values against the corresponding W yielded a straight line for most of the coordinates for each paint.

The equation for the straight line is

(W - W¹) / (C - C¹) = a + bW

By setting up two series of simultaneous equations for each paint, constants a and b can be obtained. Substituting these values in the general equation permits the calculation of weight for any desired contrast ratio. A sample set of calculations for the white paint is given below.

TYPICAL CALCULATION

(W - W¹) / (C - C¹) = a + bW (1)

W¹ = 14.6 C¹ = 0.912 (2)

(W - 14.6) / (C - 0.912) = a + bW (3)

Substituting data from Table II gives six equations

35.8 = a + 3.78b 128.7 = a + 16.4b  
41.9 = a + 4.16b 133.2 = a + 18.2b  
82.0 = a + 10.50b 148.4 = a + 19.8b  
159.7 = 3a + 18.44b 410.3 = 3a + 54.4b (4)

The equations

159.7 = 3a + 18.44b  
410.3 = 3a + 54.4b (5)

obtained by the addition of the two sets of equations above, are solved simultaneously to yield

a = 10.2  
b = 6.98

Substituting in the general equation,

C = (W - 14.6) / (10.2 + 6.98W) + 0.912 (6)

which is the contrast ratio curve equation for the white paint.

A check in the cases where no straight line was obtained suggested that the deviation was due to the fact that the set of coordinates used was out of line in relation to the other coordinates. In order to correct the deviations as much as possible, the rectification was carried out with the set of coordinates that were most dependable—i.e., weight equal to 0—and the contrast ratio of the brushout sheet. Constants a and b were calculated for each paint using the coordinates W¹ = 0, C¹ = 0.043, as outlined in the typical calculation above.

These constants were used in the hiding power equation

C = (W - 0) / (a + bW) + 0.043

and C was calculated for each W. These figures were tabulated in Tables II and III. The observed and calculated contrast ratios were plotted against weight in Figures 2 and 3. In every case, calculated contrast ratios gave a smooth curve which seemed to align the observed data properly.

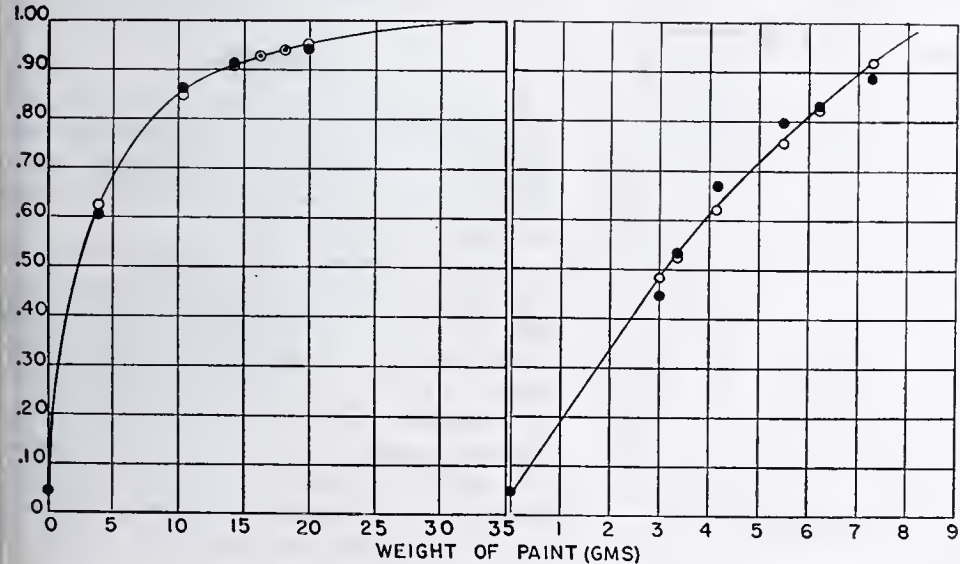


Figure 2. Rectification of Contrast Ratio Curves  
●. Observed data ○. Calculated data  
Left, white paint 1. Right, chrome green paint 2



Table II. Contrast Ratio Data for White Paint 1

W	C	C - 0.043	$\frac{W - 0}{C - 0.043}$	$\frac{\text{Caled.}}{C}$
0	0.043	...	...	...
3.78	0.610	0.567	6.66	0.628
10.5	0.862	0.819	12.82	0.855
14.6	0.912	0.869	16.80	0.910
16.4	0.926	0.883	18.57	0.926
18.2	0.939	0.896	20.31	0.939
19.8	0.947	0.904	21.90	0.949

In order to test the equation in the higher range of contrast ratios, above 0.98, the rectification was applied to data from the Baltimore Club (9). Two paints were chosen, one in which the contrast ratio was determined up to 0.990, and the other where the highest contrast ratio was 0.999. The rectification data are tabulated in Table IV and the contrast ratio curves presented in Figure 4. The equation showed excellent adherence to the data, with negligible differences between observed and calculated contrast ratios.

In brief review, the method for determining dry hiding power is as follows:

The contrast ratio of the standard black and white hiding power sheet is measured by a suitable photoelectric instrument, using a daylight filter.

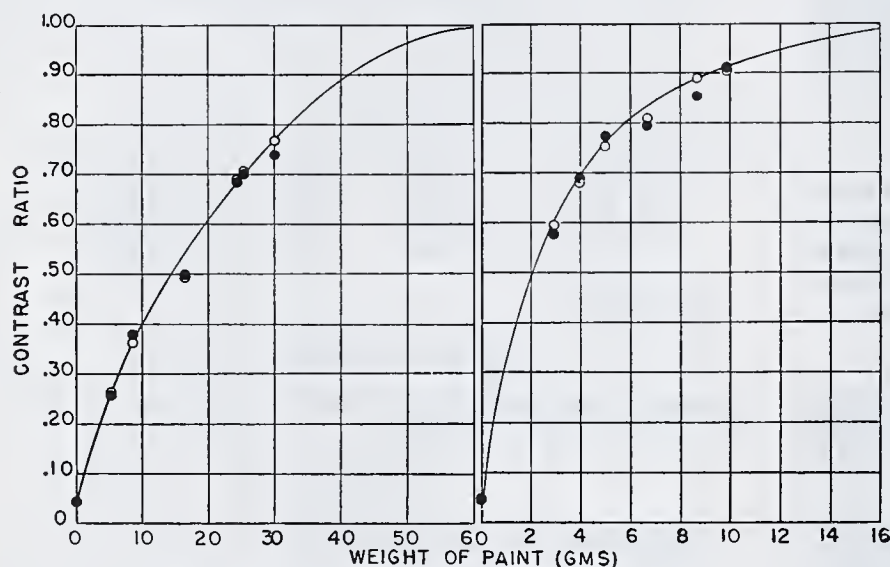


Figure 3. Rectification of Contrast Ratio Curves

●. Observed data. ○. Calculated data  
Left, Burgundy lake paint 4. Right, zinc chromate paint 3

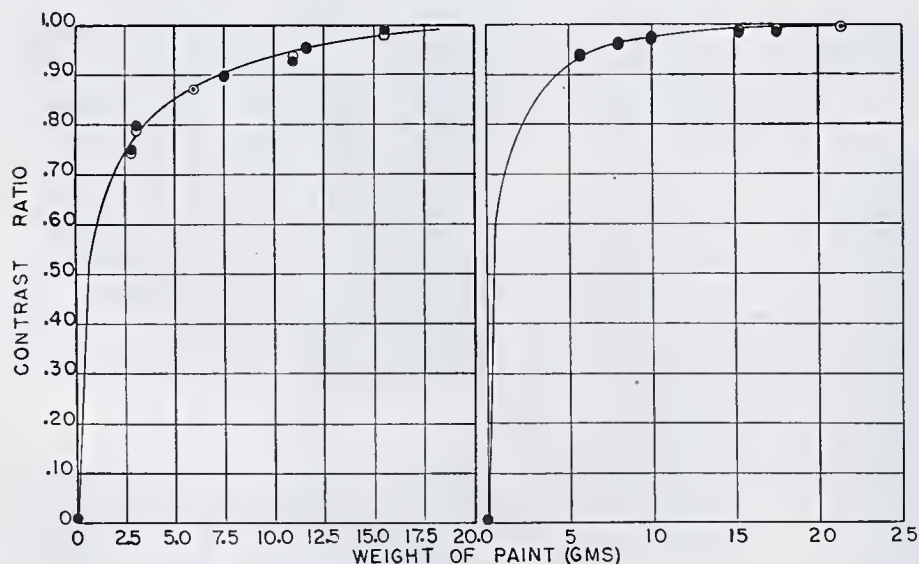


Figure 4. Rectification of Contrast Ratio Curves

●. Observed data. ○. Calculated data  
Left, titanated lithopone paint. Right, high dry hiding lithopone paint

Table III. Contrast Ratio Data

W	C	C - 0.043	$\frac{W - 0}{C - 0.043}$	$\frac{\text{Caled.}}{C}$
Chrome Green Paint 2				
0	0.043	...	...	0.043
3.0	0.445	0.402	7.46	0.481
3.4	0.533	0.490	6.94	0.531
4.2	0.672	0.629	6.67	0.623
5.5	0.792	0.749	7.35	0.759
6.2	0.830	0.787	7.87	0.825
7.3	0.884	0.841	8.68	0.923
Zinc Chromate Paint 3				
0	0.043	...	...	0.043
2.9	0.572	0.529	5.49	0.593
3.9	0.681	0.638	6.12	0.677
5.0	0.770	0.727	6.88	0.746
6.6	0.789	0.756	8.73	0.818
8.6	0.842	0.854	10.06	0.881
9.8	0.914	0.871	11.25	0.909
Burgundy Lake Paint 4				
0	0.043	...	...	0.043
5.5	0.248	0.205	26.81	0.265
8.6	0.379	0.336	25.60	0.364
13.6	0.500	0.457	29.73	0.491
24.5	0.685	0.642	38.15	0.692
25.4	0.694	0.651	39.00	0.705
30.2	0.739	0.696	40.80	0.767

Six brushouts are made on the hiding power sheets and allowed to dry for 24 hours.

The reflectances of the center squares are measured and the results averaged. A variation of more than 2% indicates a poor brushout and a new brushout is made. The average reflectance over black is divided by that over white to give the contrast ratio.

Values for  $\frac{W - W^1}{C - C^1}$  are determined using the coordinate  $W^1 = 0$ ,  $C^1$  being the contrast ratio of the unpainted brushout sheet. These values are substituted in the equation

$$\frac{W - W^1}{C - C^1} = a + bW$$

to give six equations.

The six equations are divided into two sets and added to give two equations which are solved simultaneously for constants  $a$  and  $b$ .

The hiding power equation

$$C = \frac{W}{a + bW} + C^1$$

where  $C^1$  is the contrast ratio of the unpainted hiding power sheet is solved for that value of  $C$  which is considered to indicate complete hiding. Thus value can be converted to square feet of coverage per gallon by using the equation

$$\text{H.P. (sq. ft. per gal.)} = \frac{454 \times W \text{ per gal.}}{\text{grams of paint per sq. ft.}}$$

## DISCUSSION

The measurement of hiding power has always been a controversial subject. In part, this can be attributed to the difficulty of applying uniform films of paint and misunderstanding as to what constitutes complete hiding.

The theoretical conception of the contrast ratio curve is that it is asymptotic to unit contrast ratio and that there must always be a difference between the reflectance over white and the reflectance over black, even though that difference be immeasurable. However, in making the reflectance measurements, we must use an instrument with definite sensitivity limitations. The data obtained must necessarily be bound by the limitations of the instrument used. Therefore, any empirical equation evolved from such data will represent a contrast ratio curve as developed by the reflectometer used. It will be seen



Table IV. Rectification Data

W	C	C - 0.0102	$\frac{W - 0}{C - 0.0102}$	$\frac{\text{Caled.}}{C}$
Titanated Lithopone (9, p. 29)				
0	0.0102	.....	.....	0.0102
3.2	0.750	0.7398	4.325	0.747
3.8	0.798	0.7878	4.825	0.784
6.0	0.868	0.8578	6.995	0.868
7.5	0.897	0.8868	8.475	0.900
10.9	0.936	0.9258	11.77	0.947
11.7	0.952	0.9418	12.42	0.953
15.5	0.990	0.9798	15.82	0.978
High Dry Hiding Lithopone (9, p. 29) 60% P/NV				
0	0.0102	.....	.....	0.0102
5.7	0.938	0.9278	6.13	0.943
8.0	0.965	0.9548	8.38	0.963
10.0	0.976	0.9658	10.36	0.975
15.2	0.988	0.9778	15.54	0.990
17.6	0.993	0.9828	17.90	0.994
21.4	0.999	0.9888	21.62	0.999

Table V. Data for Comparison with Fell Formula

Weight	Reciprocal of Weight	Observed Contrast Ratio	Log of 10 X Observed Contrast Ratio	Calculated Contrast Ratio	Log of 10 X Calculated Contrast Ratio
White 1					
3.78	0.264	0.610	0.7853	0.628	0.7980
10.5	0.0952	0.862	0.9355	0.855	0.9320
14.6	0.0685	0.912	0.9600	0.910	0.9590
16.4	0.0610	0.926	0.9666	0.926	0.9666
18.2	0.0550	0.939	0.9727	0.939	0.9727
19.8	0.0505	0.947	0.9763	0.949	0.9773
Chrome Green 2					
3.0	0.333	0.445	0.6484	0.481 <sup>a</sup>	0.6821
3.4	0.294	0.533	0.7267	0.531	0.7251
4.2	0.238	0.672	0.8274	0.623	0.7945
5.5	0.182	0.792	0.8987	0.759	0.8802
6.2	0.161	0.830	0.9191	0.825	0.9165
7.3	0.137	0.884	0.9465	0.923	0.9652
Zinc Chromate 3					
2.9	0.345	0.572	0.7574	0.593	0.7731
3.9	0.256	0.681	0.8331	0.677	0.8306
5.0	0.200	0.770	0.8865	0.746	0.8727
6.6	0.152	0.789	0.8971	0.818	0.9128
8.6	0.116	0.842	0.9253	0.881	0.9450
9.8	0.102	0.914	0.9609	0.909	0.9586
High Dry Hiding Lithopone (9, p. 29) 60% P/NV					
5.7	0.175	0.938	0.9722	0.943	0.9745
8.0	0.125	0.965	0.9845	0.963	0.9836
10.0	0.100	0.976	0.9894	0.975	0.9890
15.2	0.0658	0.988	0.9948	0.990	0.9956
17.6	0.0568	0.993	0.9969	0.994	0.9974
21.4	0.0467	0.999	0.9996	0.999	0.9996

<sup>a</sup> From Table III.

that in the author's equation, a value can be obtained for contrast ratio of unity. From a practical standpoint, this value represents the point at which the instrument used can no longer register difference between reflectance over white and black, and may be considered complete hiding for that instrument.

There has been little attempt made to apply hiding power equations to coatings other than white, perhaps because theoretically agreement is not to be expected except by spectrophotometric analysis and separate treatment for various wave lengths of the visible spectrum. After the present work was finished, the attention of the authors was drawn to the Fell equation (24) which shows a relationship between the log of 10 X contrast ratio and the reciprocal of the film thickness. This equation has been shown to hold for several white paints, and it is of interest to see whether this relation applies as well as the hyperbolic equation proposed here to the same data including chromatic as well as white paints. Figures 5 and 6 and Table V show this comparison; the agreement with the Fell equation is generally good. Indeed, for one of the chromatic paints (chrome green, Figure 5,

lower) the agreement is slightly better than with the hyperbolic form, since a straight line may be drawn which fits the observed data slightly more closely than the curve which corresponds to the hyperbola fitted by the authors' method. It is probable, therefore, that an alternate method for finding conveniently the weight corresponding to complete hiding could be worked out for the Fell equation, though the authors have not tried to do this.

CONCLUSION

The method for measuring dry hiding of pigmented coatings as outlined offers the means for eliminating many of the disadvantages now encountered. Its results are reproducible and it permits the operator to detect and discard poor brushouts. The use of the hiding power equation gives its reading on the paint in the state in which the information is of the most value, the dried film. The equation offers the possibility of standardizing on a value for complete hiding which would be less open to dispute than the values now used.

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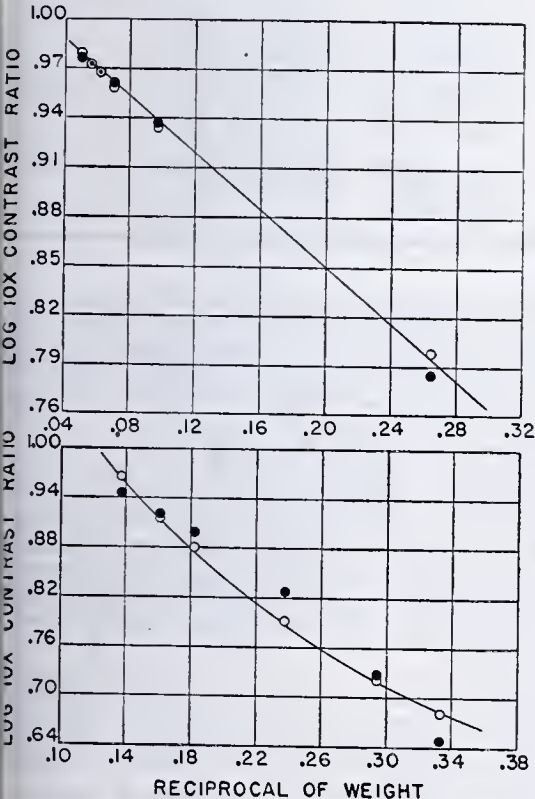


Figure 5. Comparison with Fell Equation  
●. Observed data    ○. Calculated data  
Above, white paint 1. Below, chrome green paint 2

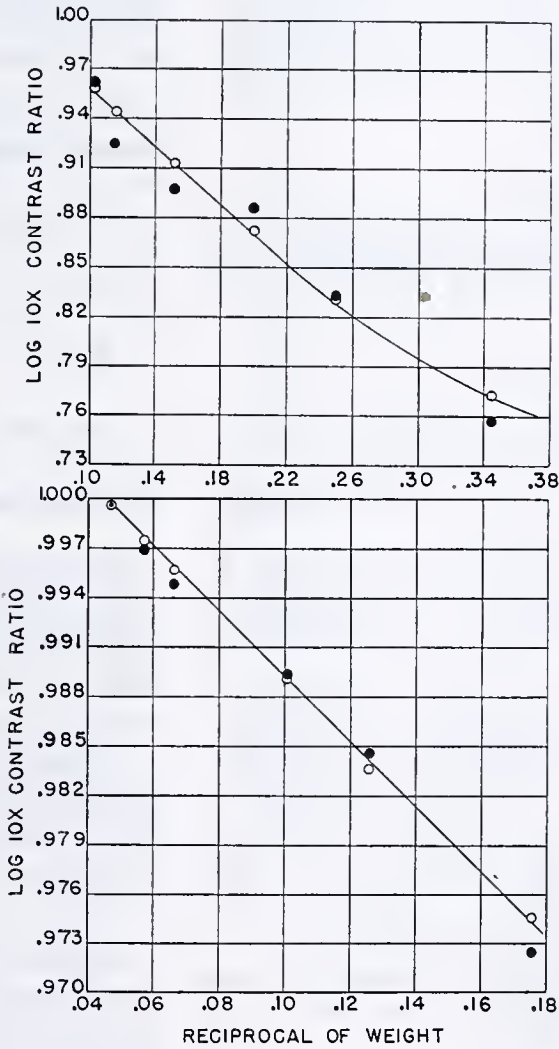


Figure 6. Comparison with Fell Equation  
●. Observed data    ○. Calculated data  
Above, zinc chromate paint 3. Below, high dry hiding lithopone paint



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# Determination of Nitrate, Nitrite, and Ammonium Nitrogen

## Rapid Photometric Determination in Soil and Plant Extracts

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THE determination of soluble inorganic nitrogen fractions in plant and soil extracts is extremely useful in explaining crop growth. In a previous paper (4) a method was described for the determination of nitrate nitrogen. The present paper gives a more rapid method for determining nitrate nitrogen and in addition, proposes methods for determination of nitrite and ammonium nitrogen in the same extract.

### APPARATUS

Photoelectric colorimeter. The amounts of reagents and samples are based on the use of the Fisher electrophotometer.

Vials (4), stirring rods, Waring Blendor, balance, oven, and 1- and 5-ml. pipets.

### REAGENTS AND SOLUTIONS

All reagents should be of C.P. grade.

EXTRACTIONS. Morgan's universal extracting solution (2), acetic acid (0.5 N), buffered at pH 4.8 with sodium acetate.

DETERMINATION OF NITRATE NITROGEN. Brucine (Merck), 1% in concentrated sulfuric acid, is prepared just prior to using. The sulfuric acid should be free of nitrates.

Nitrate nitrogen standard. Sodium nitrate, 0.0910 gram in 1000 ml. of extracting solution to supply 15 p.p.m. of nitrogen.

DETERMINATION OF NITRITE NITROGEN. Dimethylaniline (Eastman 97), 10 ml., in 1 to 6 hydrochloric acid. Concentrated hydrochloric acid.

Nitrite nitrogen standard. Potassium nitrite, 0.1210 gram in 1000 ml. of extracting solution to supply 20 p.p.m. of nitrogen.

DETERMINATION OF AMMONIUM NITROGEN. To make Graves reagent (5), 80 grams of sodium chloride are dissolved in 130 ml. of water and 100 ml. of a cold saturated solution of mercuric chloride (7%) are added with shaking. The salt is almost dissolved and 70 ml. of a saturated solution of lithium carbonate (1%) are added in small quantities and with continued shaking. Five grams of talc are added to the solution, which is filtered. Stored in a brown bottle and kept stoppered, it will keep for several weeks, but needs to be thoroughly shaken before use.

Gum arabic, 0.25%; sodium hydroxide, 15%.

Standard nitrogen. Ammonium chloride, 0.1521 gram in 1000 ml. of extracting solution to supply 40 p.p.m. of nitrogen.

### METHODS

PREPARATION OF SOIL AND PLANT EXTRACTS. Detailed directions for preparing extracts have been given (4).

CALIBRATION OF STANDARD CURVES. Aliquots of standard solutions are diluted with extracting solution to appropriate level and treated as in the determination of nutrients of soil and plant extracts. Photometer readings are taken using the appropriate filter (Table I). Deflection concentration curves are drawn from the resultant data.

Table I. Determination of Inorganic Nitrogen Fraction for Standard Curves and in Soil and Plant Extracts

Nitrogen Fraction	Material	Useful Range P.p.m.	Volume of Aliquots Ml.	Diluted to Ml.
Nitrate N	Standard soln.	0-2	0-2	15
	Soil extract	0.6-12	2.5	
	Plant extract	0.6-12	2.5	
Nitrite N	Standard soln.	0-5	0-5	20
	Soil extract	2-10	10	
	Plant extract	2-10	10	
Ammonium N	Standard soln.	0-5	0-25	20
	Soil extract	4-80	5	
	Plant extract	4-80	5	

DETERMINATION OF NUTRIENTS IN SOIL AND PLANT EXTRACTS. *Nitrate Nitrogen in the Absence of Nitrite Nitrogen.* Soil or plant extracts (2.5 ml.) are diluted to 15 ml. with extracting solution and 7.5 ml. of brucine reagent are added cautiously down the side of tube. The contents are immediately stirred with a flat-bottomed rod. Photometer readings are taken after 15 minutes, using a 425 blue filter and adjusting the blank to 100.

*Nitrate Nitrogen in the Presence of Nitrite Nitrogen.* Nitrite nitrogen will produce color changes similar to those produced by nitrate nitrogen with the brucine reagent. A concentration of nitrite nitrogen is approximately 3 times as effective as one of nitrate nitrogen, and so 3 p.p.m. of nitrate nitrogen can be subtracted for every p.p.m. of nitrite nitrogen present in the extract (for photometer readings 100 to 35). Nitrite nitrogen is determined by the method outlined blow.

For more exact determination, standard amounts of nitrite nitrogen (0.1 to 1.0 p.p.m.) are treated with brucine as for the determination of nitrate nitrogen and a standard curve is drawn from the resultant data. The amount of nitrate nitrogen equivalent to the nitrite nitrogen present is calculated from the standard curves and deducted from the total amount of nitrate nitrogen in the extract as shown by the brucine test.

*Nitrite Nitrogen.* Dimethylaniline solution (0.5 ml.) is added to each tube containing 10 ml. of soil or plant extract diluted to



0 ml. with extracting solution. The contents are stirred, 1 ml. of concentrated hydrochloric acid is added, and contents are stirred again. Readings are taken in 15 minutes, using a 425 blue filter and adjusting the blank to 100.

**Ammonium Nitrogen.** Five milliliters of soil or plant extract are diluted to 20 ml. with extracting solution and 0.2 ml. of gum arabic solution is added. The contents are mixed thoroughly by means of a flat-bottomed rod, and 0.5 ml. of Graves' reagent is added. The contents are again mixed and 3.5 ml. of 15% sodium hydroxide are added to each tube, mixing immediately after the addition of the hydroxide. Readings are taken after 5 minutes, using a 425 blue filter and a null adjustment giving a reading of 100 with the blank.

DISCUSSION OF METHODS

A blank should be run on all determinations. It is convenient to run about 12 samples at one time. Blocks to hold 13 vials (12 samples, 1 blank) facilitate handling.

The use of 7.5 ml. of brucine reagent for 15 ml. of sample plus extracting solution determines both nitrate and nitrite nitrogen. If proportionately larger amounts of sulfuric acid were used, nitrates alone would be determined (3). However, higher concentrations of sulfuric acid cause browning of organic constituents. The effects of nitrite nitrogen can also be eliminated by the use of sodium azide (1). It is simpler to use a low concentration of acid and to compensate for the amount of nitrite nitrogen present.

For many soil and plant extracts the nitrite nitrogen content will be negligible. In such cases, it can be ignored, and the results obtained from the brucine test can be considered as indicative of the nitrate nitrogen content.

The determination of nitrate nitrogen is influenced by the concentration of acid present. If the acid is added directly to the diluted extract insufficient color is produced for the range of nitrogen desired. Higher concentration of acid which would correct this situation is unsatisfactory. If the brucine reagent is added down the sides of the tube, it is possible to use sufficient concentrations of acid without browning of the organic constituents. The contents are mixed and thereby the relative concentration of acid is reduced, so that the organic constituents do not turn brown. It is important, therefore, to add the acid at a fairly uniform rate down the sides of the tube and to mix the contents immediately with a flat-bottomed rod.

In the nitrite nitrogen determination the color is influenced by the concentration of hydrochloric acid used and by the amounts of dimethylaniline. It is important to measure these reagents carefully.

A small error is introduced in the nitrite nitrogen determination because of slight color that is present in the extracts or appears upon the addition of hydrochloric acid. The test is, therefore, not accurate for less than 1 p.p.m. of nitrite nitrogen in the soil or plant extract. It is best to ignore readings for less than this amount of nitrite nitrogen.

In the ammonium nitrogen determination, the precipitate formed is influenced by the length of standing and by the gum arabic added. If allowed to stand much longer than 5 minutes the colloidal precipitate in some cases will settle out and make the readings inaccurate. Repeatable results can be obtained by taking readings exactly 5 minutes after addition of the sodium hydroxide.

The use of 0.2 ml. of gum arabic aids in the suspension of the colloidal precipitate and permits determination of larger amounts of ammonium nitrogen. The Graves' reagent may settle out on standing. If shaken to obtain a uniform suspension before using, it may be used for several weeks after preparation.

The Graves' reagent should be added after the gum arabic has been thoroughly mixed with the contents of the tube. The con-

Table II. Recovery from Mixtures of Standard Solution and of Soil and Plant Extracts Plus Standard Solutions

Nitrogen Fraction	Nitrate Nitrogen			Nitrite Nitrogen			Ammonium Nitrogen		
	Found P.p.m.	Calcd. P.p.m.	Diff. P.p.m.	Found P.p.m.	Calcd. P.p.m.	Diff. P.p.m.	Found P.p.m.	Calcd. P.p.m.	Diff. P.p.m.
Mixed solution	3.6	4.0	-0.4	2.0	2.0	0	6.8	6.0	+0.8
Plant extract	5.2	...	...	0	...	...	4.8	...	...
Plant extract and mixed solution	5.0	4.6	+0.4	1.4	1.0	+0.4	5.6	5.4	+0.2
Soil extract	1.3	...	...	1.0	...	...	4.8	...	...
Soil extract and mixed solution	2.6	2.6	0	1.6	1.5	+0.1	5.2	5.4	-0.2

tents should again be mixed immediately after addition of the Graves' reagent and mixed thoroughly after addition of sodium hydroxide.

**ACCURACY.** The nitrate nitrogen determination can be repeated within 0.1 p.p.m. for amounts of 0.1 to 2.0 p.p.m. of nitrate nitrogen. Nitrite nitrogen must be determined and deductions made accordingly to evaluate properly the nitrate nitrogen content, although the nitrite content of most plant and soil extracts can be ignored for practical purposes. The nitrite nitrogen determination can be repeated within 0.2 p.p.m. for 1 to 5 p.p.m. of nitrogen.

The ammonium nitrogen determination can be repeated within 0.2 p.p.m. for between 1 to 5 p.p.m. of nitrogen.

Where recommended aliquots are used, these variations represent 1.09 kg. (2.4 pounds) of nitrate nitrogen, 0.68 kg. (1.5 pounds) of nitrite nitrogen, and 3.63 kg. (8 pounds) of ammonium nitrogen per 908,000 kg. (2,000,000 pounds) of soil, and 24 p.p.m. of nitrate nitrogen, 8 p.p.m. of nitrite nitrogen, and 32 p.p.m. of ammonium nitrogen in plant material on a fresh weight basis.

The amounts determined in a soil and plant sample along with the amounts recovered from samples and mixed solutions are given in Table II. In general, recoveries from mixed solutions or from additions to soil or plant extracts are good.

CONCLUSIONS

Rapid photometric methods for the determination of nitrate, nitrite, and ammonium nitrogen in soil and plant extracts are given. Only one extraction of soil or plants is made, using one extracting solution for all determinations. Determinations are made on separate aliquots of the extract.

Nitrate plus nitrite nitrogen is determined by brucine reagent, nitrite nitrogen by dimethylaniline reagent. Nitrate nitrogen is obtained by difference (nitrate plus nitrite N - nitrite N = nitrate N). Ammonium nitrogen is determined by means of Graves' reagent. By careful attention to details, fairly accurate values can be obtained.

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# Determination of Antimony in Tin-Base Alloys

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The usual methods for determination of antimony in tin-base alloys yield low results in the presence of copper, due to catalyzed oxidation of antimony during solution of the sample in hot sulfuric acid solution (Low method); or to co-oxidation of antimony with copper following reduction with sulfurous acid (Rowell method). The low results can be eliminated by using a Low-Rowell method in which the sample is dissolved in hot sulfuric acid and the traces of pentavalent antimony are reduced in hydrochloric acid solution with sulfurous acid under conditions where the amount of copper reduced is too small to cause appreciable co-oxidation of antimony.

THE two methods most often used for the determination of antimony in tin-base alloys are those in which the sample is dissolved in hot sulfuric acid (Low's method, 9), or in hydrochloric acid and bromine followed by reduction of antimony and arsenic to the trivalent state with sulfurous acid (Rowell's method, 2, 15); the arsenic is expelled from strong hydrochloric acid solution by boiling; and the antimony is titrated with bromate by the method of Györy (4). (These are referred to below as the modified Low and Rowell methods.) When the arsenic content is known and is small compared to that of the antimony it is sometimes simpler to titrate both the antimony and arsenic and then correct for the latter. This is usually done by solution of the alloy in sulfuric acid, dilution with water and hydrochloric acid, and titration at 10° C. with permanganate.

The above methods are satisfactory in the absence of such interfering elements as iron and copper. These elements are usually present, however, and their effects must be considered. Iron in small quantities is not harmful in the modified Low method, since the iron is oxidized to the trivalent state in the hot sulfuric acid (16). Similarly, as Rowell has shown (15), in the Rowell method the reduction of iron in strong hydrochloric acid with sulfurous acid is slow and thus its deleterious effect is not too great. Iron in large quantities causes high results for antimony in both methods and separation must be resorted to. Fortunately, the iron content of most tin-base alloys is not high enough to cause trouble.

Copper will cause high results if it is in the cuprous state at the time of the antimony titration. On the other hand, copper, when present in amounts greater than 0.1%, may cause the results to be low, owing to its effect in catalyzing the oxidation of antimony previous to titration. This catalytic effect is usually small and can be ignored in most routine work, but in accurate work it cannot be ignored. The usual practice in such cases is to remove the copper before reducing and titrating the antimony. Because the methods of separation available are time-consuming (1), it appeared desirable to investigate the catalytic effect of the copper in the hope of finding conditions under which the direct determination can be employed without interference from the copper.

This investigation has resulted in the development of a method which yields satisfactory results in the presence of copper. The method consists essentially of dissolving the alloy in hot sulfuric acid, reducing the small amount of pentavalent antimony with sulfurous acid in strong hydrochloric acid solution, expelling arsenic and excess sulfurous acid by boiling, and titrating antimony with bromate. The method has been tested on Bureau of Standards tin-base alloys 54-a and 54-b which contain about 7% antimony, 0.05% arsenic, 3% copper, and 0.03% iron. The results are very satisfactory (see Table VI).

## REAGENTS

STANDARD POTASSIUM BROMATE SOLUTION (0.1 N). Twice recrystallize potassium bromate from water, and dry at 180° C. to constant weight. Dissolve 2.784 grams of the potassium bromate in water and dilute to 1 liter in a volumetric flask.

METHYL ORANGE SOLUTION. Dissolve 0.1 gram of methyl orange in 100 ml. of water.

## PROCEDURE

Transfer 1 gram of the finely divided sample (free of metallic iron) into a dry 500-ml. Erlenmeyer flask. Add 10 ml. of sulfuric acid and heat without cover first on a hot plate and then on a Tirrill flame until complete dissolution of the sample is attained, and copious white fumes are being evolved. During the initial treatment with acid avoid heating the sample on a plate that is too hot; otherwise the sample may melt and complete dissolution will become very difficult. Cool. Add 10 ml. of water and 3 or 4 grains of 12-mesh silicon carbide to act as an antibump. Add 50 ml. of hydrochloric acid and warm the solution for a few minutes to dissolve all salts. Adjust the temperature to approximately 50° C. Add 25 ml. of sulfurous acid (6% and place the flask on a hot plate with surface temperature of 275° to 300° C. Boil the solution without cover until the volume is reduced to 60 ± 5 ml., remove from the plate, and dilute to 350 ml. with boiling water. Pass a fairly rapid stream of air or oxygen through the solution for 5 minutes. Titrate in the usual manner with 0.1 N potassium bromate solution, using methyl orange as indicator:

$$(\text{Ml. of KBrO}_3 - \text{blank}) \times 0.609 = \% \text{ antimony}$$

## DISCUSSION

CATALYTIC OXIDATION OF ANTIMONY IN PRESENCE OF COPPER. Copper has been used as catalyst in the reduction of arsenic and antimony (3, 10). On the other hand, it appears that under certain conditions copper can also act as catalyst in the oxidation of the two metals. Thus, it has been shown that low results for antimony are obtained in the Low or Rowell methods for the analysis of lead and tin alloys when the latter contain copper (6, 16, 17). Rowell and other workers have reported high results for antimony when copper is present, but it is probable that in these instances precautions were not taken to ensure complete oxidation of the copper to the cupric state by bubbling air through the solution before titration of the antimony.

The author has recently studied the behavior of copper in the analysis of antimony in tin-base alloys by the Rowell and modified Low methods. Experiments indicate that in the Rowell method the low results are caused by co-oxidation of antimony with cuprous ion as the latter is oxidized by air or oxygen previous to the bromate titration. Cupric ion does not catalyze the oxidation of antimony in hydrochloric acid solution (17) (see Tables I and II).

Table I. Co-Oxidation of Antimony with Copper

No.	Copper Added Mg.	Time of Bubbling Min.	KBrO <sub>3</sub> Used <sup>a</sup> Ml.
1 <sup>b</sup>	.....	0	13.70
2 <sup>b</sup>	.....	0	13.73
3	.....	5	13.73
4	.....	5	13.71
5	30 Cu <sup>++</sup>	5	13.70
6	60 Cu <sup>++</sup>	10	13.73
7	120 Cu <sup>++</sup>	10	13.74
8	60 Cu <sup>+</sup>	5	13.62
9	120 Cu <sup>+</sup>	10	13.40
10	120 Cu <sup>+</sup>	10	13.46
11	120 Cu <sup>+</sup>	10	13.45

<sup>a</sup> Exact amount of Sb in aliquot unknown.

<sup>b</sup> Solution was not bubbled with oxygen.



Table II. Co-Oxidation of Antimony with Copper			
No.	Copper Added	HCl Added	Antimony Found <sup>a</sup>
	Mg.	Ml.	
1 <sup>b</sup>	...	15	1.0
2 <sup>b</sup>	120	15	1.0
3 <sup>b</sup>	...	50	0.2
4 <sup>b</sup>	120	50	0.2
5 <sup>c</sup>	...	15	50.0
6 <sup>c</sup>	...	15	50.0
7	...	15	50.1
8 <sup>d</sup>	...	15	49.9
9 <sup>d</sup>	30	15	49.4
10 <sup>d</sup>	120	15	49.3
11	120	15	49.1
12	120	15	49.4
13	...	50	50.1
14	...	50	50.0
15	120	50	50.0
16	120	50	49.9
17	120	50	50.0
18 <sup>e</sup>	...	50	50.0
19 <sup>e</sup>	...	50	50.1
20 <sup>e</sup>	30	50	49.2
21 <sup>e</sup>	60	50	48.9

<sup>a</sup> Antimony present, 50.0 mg.  
<sup>b</sup> Blank carried through entire procedure.  
<sup>c</sup> Samples titrated without treatment with Na<sub>2</sub>SO<sub>3</sub>.  
<sup>d</sup> Antimony titrated with 0.1 N KBrO<sub>3</sub> just before treatment with Na<sub>2</sub>SO<sub>3</sub>.  
<sup>e</sup> 10 ml. of HCl-Br<sub>2</sub> solution (100 ml. of HCl + 12 ml. of Br<sub>2</sub>) added just before reduction with Na<sub>2</sub>SO<sub>3</sub>. H<sub>2</sub>SO<sub>3</sub> (6% solution) used in place of water and dilution to 100 ml.

From the foregoing it is evident that it should be possible to obtain correct results for antimony in the Rowell method if conditions can be found for completely reducing antimony with sulfurous acid without reducing the copper. It appeared that this might be accomplished by complexing the copper with hydrochloric acid. Experiments showed that reduction of copper with sulfurous acid is very slow and incomplete in a strong hydrochloric acid solution, where the copper may exist in the form of complex acids (11). The small amount of cuprous ion which is produced induces very little co-oxidation of antimony during the oxidation of the former with oxygen previous to the bromate titration (see Table II).

Unfortunately, however, it was found that the rate of reduction of pentavalent antimony with sulfurous acid is, like that of copper, very slow in strong hydrochloric acid solution. Bromide catalyzes the reaction and permits rapid quantitative reduction of antimony in strong acid solution, but appears to catalyze the reduction of copper in the same manner, with the result that co-oxidation of the antimony takes place (see Tables III and IV).

Table III. Reduction of Antimony with Sulfurous Acid			
No.	Oxidant Used	HCl Added	Antimony Found <sup>a</sup>
		Ml.	
1	KBrO <sub>3</sub>	15	50.0
2	KMnO <sub>4</sub>	15	49.9
3	Br <sub>2</sub>	15	50.1
4	KClO <sub>3</sub>	50	14.3
5	KClO <sub>3</sub>	50	13.6
6 <sup>b</sup>	KClO <sub>3</sub>	50	8.0
7 <sup>c</sup>	KClO <sub>3</sub>	50	11.9
8	KMnO <sub>4</sub>	50	15.3
9	H <sub>2</sub> O <sub>2</sub>	50	9.2
10 <sup>d</sup>	Br <sub>2</sub>	50	46.0
11 <sup>d</sup>	Br <sub>2</sub>	50	48.1
12	Br <sub>2</sub>	50	50.0
13	Br <sub>2</sub>	50	50.1
14	Br <sub>2</sub>	50	49.9
15 <sup>b</sup>	Br <sub>2</sub>	50	48.9

<sup>a</sup> Antimony present, 50.0 mg.  
<sup>b</sup> 120 mg. of copper as CuSO<sub>4</sub>·5H<sub>2</sub>O added before oxidant.  
<sup>c</sup> Only about 95% of antimony was oxidized with bromate.  
<sup>d</sup> After addition of acid excess Br<sub>2</sub> was expelled by boiling previous to reduction with sulfite.

In the modified Low method copper catalyzes the oxidation of antimony and arsenic during solution of the sample in hot sulfuric acid. This oxidation does not appear to be associated with cuprous ion, for the amount of oxidation is proportional to the time of heating (17). Recent work has shown that the amount of oxidation is also controlled by the concentration of the sulfuric acid used in the solution of the alloy. Thus, when a 1-gram

Table IV. Determination of Antimony in Tin-Base Alloys by Rowell Method			
No.	Sample	Oxidant Used	Antimony Found <sup>a</sup>
			%
1	54-b	KClO <sub>3</sub>	6.08
2	54-b	KClO <sub>3</sub>	6.20
3 <sup>b</sup>	54-b	KClO <sub>3</sub>	6.60
4	54-b	Br <sub>2</sub>	7.35
5	54-b	Br <sub>2</sub>	7.37
6	54-b	Br <sub>2</sub>	7.35
7 <sup>c</sup>	54-b	Br <sub>2</sub>	7.33
8	54-a	Br <sub>2</sub>	7.24
9	54-a	Br <sub>2</sub>	7.22
10	54-a	Br <sub>2</sub>	7.22

<sup>a</sup> Certificate value for 54-a = 7.32 and for 54-b = 7.39.  
<sup>b</sup> 100 mg. of KBr added just before reduction with sulfite.  
<sup>c</sup> Solution of alloy and reduction with sulfite performed under a reflux condenser.

Table V. Determination of Antimony in Tin-Base Alloys by Modified Low Method			
No.	Sample	Antimony Found <sup>a</sup>	Notes
		%	
1	54-a	7.22	Wet flask
2	54-a	7.26	Wet flask
3	54-a	7.24	Wet flask
4	54-a	7.28	Acid fumed
5	54-a	7.28	Acid fumed
6	54-b	7.32	Wet flask
7	54-b	7.37	Wet flask
8	54-b	7.35	Dry flask but acid was not fumed
9 <sup>b</sup>	54-b	7.35	Dry flask but acid was not fumed
10 <sup>b</sup>	54-b	7.36	Dry flask but acid was not fumed
11 <sup>b, c</sup>	54-b	7.39	Dry flask but acid was not fumed
12	54-b	7.40	Acid fumed
13	54-b	7.42	Acid fumed
14	54-b	7.41	Acid fumed

<sup>a</sup> Certificate value for 54-a = 7.32 and 54-b = 7.39.  
<sup>b</sup> 1 gram of KBr added before distillation of arsenic.  
<sup>c</sup> Sample treated with H<sub>2</sub>SO<sub>3</sub> as directed in Procedure.

sample of tin-base alloy is dissolved in 10 ml. of ordinary concentrated sulfuric acid to which a few drops of water have been added, rapid and complete solution of the alloy occurs and catalyzed oxidation of the antimony is pronounced. On the other hand, if the sulfuric acid has been fumed free of excess water before addition of the sample, dissolution is more difficult and may even be somewhat incomplete. The copper is precipitated as the anhydrous sulfate and causes little or no oxidation of antimony (see Table V).

Because of the difficulty of controlling conditions with various types of alloys, however, it does not appear practical to use a method which depends upon elimination of error by precipitation of the copper as sulfate. Instead, it should be better to dissolve in the presence of sufficient water to ensure complete dissolution of the alloy and then reduce the small amount of pentavalent antimony present.

It is obvious that nothing would be accomplished by attempting to reduce the antimony in a hot sulfuric acid solution with the aid of the usual carbon or sulfur compounds. It has been found that the reduction can be accomplished successfully with sulfurous acid in strong hydrochloric acid solution without appreciable reduction of copper (see Table VI). Apparently the reduction of very small amounts of antimony is complete

Table VI. Determination of Antimony in Tin-Base Alloys by Proposed Method		
No.	Sample	Antimony Found <sup>a</sup>
		%
1	54-a	7.34
2	54-a	7.30
3	54-a	7.33
4	54-a	7.31
5	54-b	7.41
6	54-b	7.42
7	54-b	7.43
8 <sup>b</sup>	54-b	7.42
9 <sup>b</sup>	54-b	7.41
10 <sup>b</sup>	54-b	7.41
11 <sup>b</sup>	54-b	7.43
12 <sup>b, c</sup>	54-b	7.41
13 <sup>c</sup>	54-b	7.41
14 <sup>d</sup>	54-b	7.42

<sup>a</sup> Certificate value for 54-a = 7.32 and 54-b = 7.39.  
<sup>b</sup> 10 mg. of As<sup>+++</sup> added before addition of H<sub>2</sub>SO<sub>3</sub>.  
<sup>c</sup> 1 ml. of water added with H<sub>2</sub>SO<sub>3</sub>.  
<sup>d</sup> 100 rather than 50 ml. of HCl added.



under conditions where highly incomplete reduction is obtained with large amounts of antimony (see Table IV, experiments 1 to 3).

From the above it would appear that in the absence of bromide it might be possible to obtain correct results in the Rowell method by reducing first in dilute acid solution and then, after oxidation of the cuprous ion, repeating the reduction in strong hydrochloric acid solution.

**REDUCTION OF PENTAVALENT ANTIMONY WITH SULFUROUS ACID.** The rate of reduction of antimony with sulfurous acid is markedly affected by the acidity of the solution. Kurtenacker and Fürstenau (8) have shown that the optimum acid concentration for the reduction of antimony in hydrochloric acid solution is about 2 *N*. The rate of reduction is at a minimum in (1 to 1) hydrochloric acid and complete reduction in such solution is attained only by repeated treatments with sulfurous acid (7). Rohmer (13, 14) found that reduction is greatly accelerated by the presence of bromide. The author has confirmed this and has shown that rapid and complete reduction of antimony in strong hydrochloric acid solution can be attained if bromide is present (see Table III).

It appears probable that pentavalent antimony chloride can exist in strong hydrochloric acid solution in the form of Werner complex acids such as  $\text{HSbCl}_6$ ,  $\text{H}_2\text{SbCl}_7$ , and  $\text{H}_3\text{SbCl}_8$  (12). With such compounds it is evident that reduction of the antimony with sulfurous acid will be difficult, since the tendency for the antimony to accept electrons and become reduced is diminished because the chlorine atoms are sharing their electrons with the antimony.

When bromide is added to the solution before reduction with sulfurous acid it is probable that there is an intermediate formation of a certain amount of the corresponding complex bromide acid. This should be less stable than the chloride acid because of the greater size of the bromine atom, and hence reduction of the antimony should be easier.

The addition of bromide should be avoided wherever possible—e.g., when reducing antimony in dilute hydrochloric acid solution—because the bromine produced in the subsequent titration with bromate reacts but slowly with the antimony and indicator, and the blank is high (see Table VII).

complete without loss of antimony on alloys of the type of Bureau of Standards tin-base alloys Nos. 54-a and 54-b.

## EXPERIMENTAL

Aliquot portions of a solution of antimony trichloride, each containing about 75 mg. of antimony, together with 20 ml. hydrochloric acid and 20 ml. of (1 to 1) sulfuric acid were diluted to 300 ml. with boiling water. Measured amounts of copper sulfate or cuprous chloride were dissolved in the solution, a rapid stream of oxygen was bubbled through the solution for 5 or 10 minutes, and the antimony was titrated with standard potassium bromate solution (0.1 *N*). The results are recorded in Table I.

Aliquot portions of a solution, each containing 50 mg. of antimony as antimony trichloride, 5 ml. of hydrochloric acid, and 5 ml. of sulfuric acid were treated in a 500-ml. Erlenmeyer flask with 15 or 50 ml. of hydrochloric acid and then diluted to 300 or 100 ml., respectively. Measured amounts of copper in the form of copper sulfate were added and the solutions were then heated to 60° C., treated with 2 grams of anhydrous sodium sulfite, boiled at a moderate rate for 10 minutes, and diluted, bubbled, and titrated as directed in the Procedure (see Table II). The results have been corrected for the blanks indicated.

The above experiment was repeated, oxidizing the antimony with a slight excess of various oxidants previous to the addition of the hydrochloric acid (see Table III).

One-gram samples of Bureau of Standards tin-base sample 54-a and 54-b (containing about 7% antimony, 0.05% arsenic, 3% copper, and 0.03% iron) were transferred into 500-ml. Erlenmeyer flasks, warmed gently (so as not to lose too much acid) with 50 ml. of hydrochloric acid until most of the sample had dissolved, and finally dissolved completely by the addition of small portions of potassium chlorate or by adding 10 ml. of hydrochloric acid-bromine mixture (12 ml. of bromine dissolved in 100 ml. of hydrochloric acid). Sufficient bromine or chlorate was added to oxidize all the tin and copper. The samples were then cooled to 50° C., and treated with 35 ml. of sulfurous acid (6%) and 2 grams of anhydrous sodium sulfite, and the antimony was then reduced and titrated as directed in the Procedure. The certificate values for antimony are 7.32% for sample 54-a and 7.39% for 54-b (see Table IV). The values given have been corrected for a blank of 0.02 ml. of 0.1 *N* potassium bromate for the samples oxidized with chlorate and 0.06 ml. for those oxidized with bromine.

Samples 54-a and 54-b were analyzed as directed in the Procedure, adding water rather than sulfurous acid previous to the distillation of the arsenic. Some of the samples were dissolved in 10 ml. of sulfuric acid containing small amounts of water. With others, the 10 ml. of sulfuric acid were heated in a 500-ml. Erlenmeyer flask to copious white fumes to expel all water, and cooled nearly to room temperature before addition of the sample (see Table V). The values shown have been corrected for a blank of 0.02 ml. of 0.1 *N* potassium bromate.

Samples 54-a and 54-b were analyzed as directed in the Procedure (see Table VI). The values recorded have been corrected for a blank of 0.02 ml. of 0.1 *N* potassium bromate.

Known amounts of antimony and arsenic as trichloride together with 10 ml. of sulfuric acid, 50 ml. of hydrochloric acid, and 2 or 50 ml. of sulfurous acid were boiled down to 50, 65, or 75 ml. in a 500-ml. Erlenmeyer flask. The samples were then diluted, bubbled, and titrated as directed in the Procedure (see Table VII).

## SUMMARY

In the modified Low or Rowell methods for the determination of antimony in tin-base alloys low results are obtained in the presence of copper due to catalyzed oxidation of antimony during solution of the sample in hot sulfuric acid (modified Low method), or to co-oxidation of antimony with cuprous ion as the latter is oxidized just before the bromate titration (Rowell method).

Attempts to eliminate the low results in the Rowell method by selective reduction of antimony with sulfurous acid have failed. Suitable conditions for quantitative reduction of antimony without appreciable reduction of copper could not be found.

The low results can be eliminated in the modified Low method by reducing the small amount of pentavalent antimony in strong hydrochloric acid solution with sulfurous acid previous to the titration. Under controlled conditions it is possible to reduce completely the small amount of pentavalent antimony without reducing sufficient copper to cause co-oxidation of antimony.

Table VII. Quantitative Removal of Arsenic by Distillation

No.	Arsenic Present Mg.	H <sub>2</sub> SO <sub>3</sub> Added Ml.	Volume after Boiling Ml.	KBrO <sub>3</sub> Used Ml.
1	10	50	75	0.20
2	1	50	75	0.09
3	0	50	75	0.04
4	10	50	65	0.09
5	0	50	65	0.02
6	0	50	65	0.03
7	10	25	65	0.04
8	10	25	65	0.03
9	10	25	50	0.03
10	0	25	50	0.02
11	0	25	65	0.02
12 <sup>a</sup>	0	25	65	0.06
13 <sup>a</sup>	0	25	50	0.06
14 <sup>b</sup>	0	25	65	8.22
15 <sup>b</sup>	0	25	65	8.24
16 <sup>b</sup>	10	25	65	8.24
17 <sup>b</sup>	10	25	65	8.23

<sup>a</sup> 1 gram of KBr added just before titration.

<sup>b</sup> 50 mg. of Sb<sup>+++</sup> added. Theoretical titer, 8.20 ml.

**EXPULSION OF ARSENIC.** Methods such as that described in the Procedure are empirical in the sense that they cannot be used indiscriminately on alloys of widely varying concentration of antimony and arsenic. Before attempting an analysis the analyst should assure himself that suitable conditions of acidity, concentration, and temperature have been chosen in order that complete expulsion of arsenic and sulfurous acid can be obtained without loss of antimony by volatilization.

A high hydrochloric acid concentration is required for rapid quantitative distillation of arsenic trichloride (see Table VII). Loss of antimony by volatilization is negligible "if the distillation is made from dilute solutions of the elements and the temperature of the vapor is held under 108° C." (5). If the concentration of antimony in the solution is too great some loss will occur even at temperatures below 108° C.

From Tables VI and VII it is seen that, under the conditions recommended in the Procedure, the distillation of arsenic is



Alternately, the deleterious effect of copper can sometimes be eliminated to a large extent in the modified Low method by dissolving the sample in prefumed sulfuric acid, whereupon the copper precipitates as the anhydrous sulfate in which form it is harmless.

Reduction of antimony, arsenic, and copper with sulfurous acid in strong hydrochloric acid solution is slow, owing to complex formation, but is greatly accelerated by the addition of stannous chloride.

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## Rapid Method for Estimation of Penicillin

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A rapid method for the estimation of penicillin is based on the observation that penicillin inhibits the production of nitrite in *Staphylococcus aureus* cultures. The test can be carried out in 60 to 90 minutes. The accuracy is greater than that of a standard serial dilution method.

THE commonly used methods for the estimation of penicillin are based on its inhibition of the multiplication of a test organism, usually *Staphylococcus aureus*. With the thought that instead of observation of the multiplication of cells, metabolic processes of these cells could serve for the estimation of antibiotic substances, the authors investigated the production of nitrite from nitrate by actively growing *Staphylococcus aureus* cultures and found that this metabolic property would lend itself to the estimation of penicillin. Since nitrite production manifests itself rapidly in an actively growing culture, penicillin could be estimated by this method in a little over an hour.

The method is based on the following facts:

Actively growing *Staphylococcus aureus* cultures produce nitrite from nitrate (Table I). When penicillin is added to such a culture the authors have observed a gradual decrease in nitrite production, probably as a result of inhibition of multiplication. Within limits the decrease in nitrite production is a function of the concentration of penicillin (Figure 1). The amount of nitrite produced can be determined by a colorimetric method.

The sensitivity of the test depends on the heaviness of the inoculum. The more concentrated the *Staphylococcus* suspension,

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the more penicillin is required to produce a definite decrease in the production of nitrite. A 1 to 4 suspension of a 24-hour culture was found satisfactory for carrying out the test in 60 to 90 minutes. In 5-cc. amounts of this bacterial suspension, 0.5 Oxford unit of penicillin will produce a definite decrease in the production of nitrite. The sensitivity of the test can be increased considerably by reducing the inoculum; however, there is a corresponding increase of the lag phase in the nitrite production and consequently a longer time is required for carrying out the test.

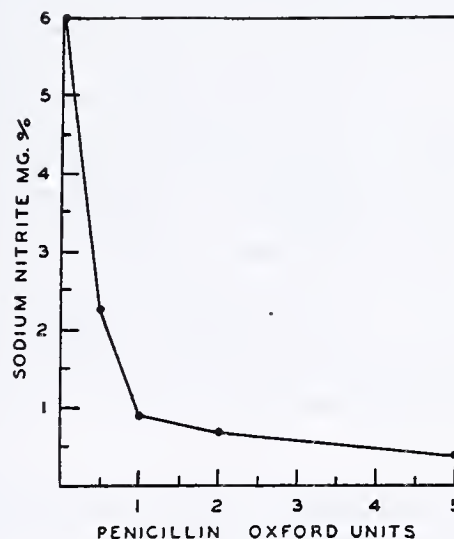


Figure 1. Effect of Penicillin on Nitrite Production by *Staphylococcus aureus*

Table I. Reduction of Nitrate to Nitrite by Suspensions of a 24-Hour *Staphylococcus aureus* Culture

Dilution of Culture	Nitrite Produced (Expressed as Sodium Nitrite)		
	60 minutes	75 minutes	90 minutes
1:4	4.3	9.5	21.6
1:4	4.6	10.1	22.4
1:5	2.8	6.9	18.0
1:5	2.8	6.8	18.0
1:6	1.9	4.3	8.6
1:6	1.9	4.0	8.2

A standard solution of calcium penicillin is tested together with the unknowns and in this manner the anti-*Staphylococcus* activity of the unknowns can be expressed in Oxford units. The standard sample of calcium penicillin containing 135 Oxford units per mg. was obtained from the Northern Regional Research Laboratories, U. S. Department of Agriculture, Peoria, Ill.

The microorganism used in these experiments was a *Staphylococcus aureus* strain isolated from a human septicemia. The



authors have also used strain No. 313 of the Northern Regional Research Laboratories, U. S. Department of Agriculture, and the H strain from the Research Laboratories of Merck and Co. All were satisfactory for carrying out the procedure.

#### EXPERIMENTAL

The test strain of *Staphylococcus aureus* is grown for 24 hours on medium No. 1, which contains 2% Difco peptone, and 0.6% sodium chloride U.S.P. This 24-hour culture is added to ice-cold medium No. 2 to make a 1 to 4 suspension. Medium No. 2 contains 2% Difco peptone, 0.6% sodium chloride U.S.P., 0.020% sodium nitrate, reagent quality, and 0.05% *p*-aminobenzoic acid Eastman.

The *Staphylococcus* suspension is plunged into ice water and after swirling is left on ice for 10 to 15 minutes. In the meantime the standard solution of penicillin and the unknowns are diluted with 0.05 molar sodium phosphate buffer to contain approximately 0.5 to 1.5 Oxford units per cc. One cubic centimeter of each solution is pipetted into 50-cc. Erlenmeyer flasks in duplicate, and 1 cc. of buffer is pipetted into each of 3 control flasks.

The phosphate buffer is prepared by mixing equal volumes of 0.05 molar mono- and dibasic sodium phosphate solutions.

Five cubic centimeters of the ice-cold *Staphylococcus* suspension are pipetted into each flask. After moderate shaking the flasks are placed in a constant-temperature water bath at 37° C. After 60 to 90 minutes one control flask is removed for the determination of nitrite. If the concentration of sodium nitrite is between 5 and 8 mg. per 100 cc. in the control, all the flasks are removed from the water bath, shaken, and immediately cooled in ice water. The concentration of nitrite is then determined. For the sake of uniform treatment all flasks are kept in a wire basket and are shaken simultaneously.

#### DETERMINATION OF NITRITE

The determination of nitrite is based on a method described by Shinn (2) with the modification that *p*-aminobenzoic acid instead of sulfanilamide is used as a primary standard.

The solution to be tested (0.5 cc.) is pipetted into a test tube, and 5 cc. of distilled water and 1 cc. of 15% trichloroacetic acid solution are added. The tubes are shaken and after 3 minutes 0.5 cc. of a 0.1% solution of *N*-(1-naphthyl)-ethylenediamine dihydrochloride is added. The color which develops reaches its maximum intensity in 3 minutes. Then 3 cc. of distilled water are added and the intensity of the dye is determined using a photoelectric colorimeter. A Cenco green filter No. 525 P was used in all determinations. Since an excess of *p*-aminobenzoic acid is present in the medium, the intensity of the color depends on the concentration of nitrite present. The concentration of diazotized *p*-aminobenzoic acid is determined from a calibration curve made in the following manner: Standard solutions of *p*-aminobenzoic acid containing 0.5, 1, 2, 5, and 10 mg. per 100 cc. are prepared, 0.5-cc. portions of each solution are pipetted into test tubes, and the following solutions are added: 5 cc. of distilled water, 1 cc. of a 15% trichloroacetic acid solution, and 0.5 cc. of a 0.1% sodium nitrite solution. The tubes are shaken. After 3 minutes 0.5 cc. of a 0.5% ammonium sulfamate solution is added, 2 minutes later 0.5 cc. of a 0.1% *N*-(1-naphthyl)-ethylenediamine dihydrochloride solution is added, 3 cc. of distilled water are pipetted into each tube, and readings are made in a photoelectric colorimeter.

To obtain the concentration of sodium nitrite in the cultures, the values for *p*-aminobenzoic acid are multiplied by the factor 0.50, which represents the ratio between the molecular weights of sodium nitrite and *p*-aminobenzoic acid.

Once the concentration of sodium nitrite is determined in all cultures, the controls and the solutions which contain 0.5, 1, and 1.5 Oxford units will give a standard curve (see Figure 1) from which the concentration of penicillin in the unknown solutions can be read in Oxford units.

Although the solutions tested in general penicillin work are not likely to contain nitrite, it is well to carry out a rapid qualitative test for nitrite in all solutions in which contamination with nitrite is suspected.

The main advantage of the method presented lies in the fact that it can be carried out more rapidly than other methods used at present. Owing to the short incubation the solutions to be

tested do not need to be passed through a bacteria filter. The principle on which this method is based could be used for the determination of other antibiotic substances which inhibit the growth of *Staphylococcus aureus*. The authors found it applicable for the determination of flavicin (1).

The accuracy of the method is very satisfactory if all cultures receive the same treatment, especially as regards temperature and time. In a typical experiment the nitrite production was measured in 20 cultures without penicillin and in 20 which contained 1 Oxford unit of penicillin per 6 cc. After 80 minutes, the mean concentration of sodium nitrite in the first series was 5 mg. per 100 cc. with a coefficient of variation of 3.4%. In the second group it was 2.5 mg. per 100 cc. with a coefficient of variation of 1.5%.

Table II. Comparison of Nitrite and Serial Dilution Assays of Standard Calcium Penicillin

Nitrite Assay		Dilution Assay	
Added	Found	Standard solution	Found
Oxford units	Oxford units	Oxford units per cc.	Dilution units per cc.
1	0.85	1.33	18.5
1	1.0	1.33	18.5
1	1.0	1.33	18.5
1	0.9	1.33	18.5
1	0.95	1.33	23.5
1	0.95	1.33	23.5
1	1.0	1.33	23.5
1	1.1	1.33	23.5
1	1.1	1.33	23.5
1	1.05	1.33	23.5
1	1.0	1.33	23.5
1	0.95	1.33	23.5
1	1.1	1.33	23.5
1	0.95	1.33	23.5
1	0.95	1.33	23.5
1	1.15	1.33	<25.0
1	1.15	Mean 1.33	22.7
1	1.10		
1	1.0		
1	1.05		
1	1.10		
Mean 1	1.015		

#### COMPARISON OF NITRITE AND SERIAL DILUTION ASSAY

Twenty "nitrite" assays and 15 dilution assays were carried out on the standard calcium penicillin solution in order to compare the variation of the two methods. For the dilution assay a solution of the standard calcium penicillin was made up to contain 1.33 Oxford units per cc., and was sterilized by passing it through a glass bacteria filter. This solution was pipetted into graduated tubes containing 5 cc. of Difco broth. The final dilutions were 1 to 16, 1 to 21, and 1 to 26. The tubes were inoculated with 0.1 cc. of a 1 to 250 dilution of a 24-hour *Staphylococcus aureus* culture. Readings were made after 24 hours' incubation and the mean value of the greatest dilution at which no bacterial growth occurred, and the lowest dilution at which the bacteria produced visible growth was arbitrarily considered as the number of dilution units per cc. (Table II).

For the nitrite assay 0.2 cc. of a standard calcium penicillin solution containing 5 Oxford units per cc. was pipetted into each of 20 Erlenmeyer flasks and the test was carried out as described above. The concentration of nitrite was determined in 80 minutes and the number of Oxford units were read from the standard curve. The results are shown in Table II.

The mean for the nitrite assay was 1.015 Oxford units with a coefficient of variation of 8.5%, and for the dilution assays was 22.7 dilution units with a coefficient of variation of > 24.0%. It is obvious that the variability was greater in the dilution assay than in the nitrite assay.

#### ACKNOWLEDGMENT

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# Determination of Freon-Insoluble Solids in Twenty Per Cent Pyrethrum Extracts

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test tube is described which permits quick determinations of the solubility of pyrethrum extracts in Freon. This tube may be used generally for solubility determinations under pressure in solvents which are gases under normal pressures.

THE bulk of the pyrethrum flowers now imported into this country is processed into extracts containing 20% pyrethrins. These extracts, after addition of sesame oil, are dissolved in Freon 12 and thus form the basis of the aerosol insecticide sprays. While the pyrethrins themselves are perfectly soluble in Freon, 20% pyrethrum extracts will vary in solubility, depending on the degree to which natural impurities insoluble in Freon have been removed and on the care exercised to avoid formation of pyrethrin polymerization products. Such products when assayed for pyrethrin content by the A.O.A.C. or Seil method may show a pyrethrin content as high as 50%, but when tested against insects, they are practically inactive. These polymerization products are insoluble in Freon. An extract containing a large proportion of insolubles therefore has less active pyrethrins available than the assay indicates.

This consideration and the obvious difficulties of handling a 20% pyrethrum extract which contains a considerable portion of insoluble solids make desirable a quick laboratory method for determining the percentage of Freon-insoluble solids. This laboratory has worked out a test procedure which gives sufficiently reproducible results to serve as a basis for judging pyrethrum extracts.

The equipment, shown in Figure 1, is simple and may be quickly assembled. A sample of 20% extract is weighed into a glass test tube, Freon is added, the tube is centrifuged to separate suspended solids, the insolubles are removed through a felt pad, the test tube is washed several times with Freon, and the dissolved portion is weighed.

TEST TUBE. *M* is a glass test tube (Scientific Glass Co., Catalog No. 100) designed to hold 100 ml.-centrifuge tubes. Through *M* two slots, *N*, have been cut on opposite sides to make the contents of the test tube *J* visible. *J* is a test tube of 2-mm. wall thickness and an outside diameter of 29 mm. which fits into *M* without too much play. It is 92 mm. long and rests in the metal

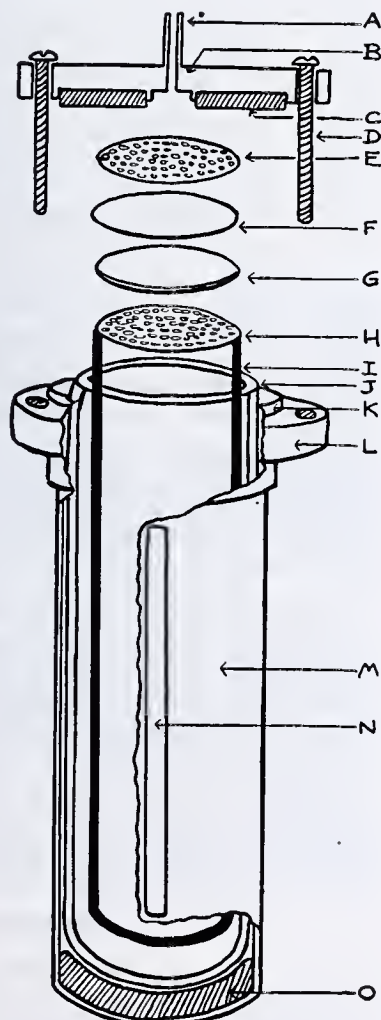


Figure 1. Equipment

Table I. Freon-Insolubles in 20% Pyrethrum Extract

Sample No.	Insolubles %	Deviation %	Sample No.	Insolubles %	Deviation %
1	5.61	0.31	15	1.98	0.05
2	5.30			2.03	
3	3.25	0.21	16	1.89	0.02
	3.46			1.87	
4	4.44	0.06	17	0.47	0.01
	4.38			0.46	
5	3.50	0.03	18	2.22	0.09
	3.47			2.31	
6	4.14	0.11	19	0.34	0.01
	4.03			0.33	
7	3.09	0.01	20	3.54	0.12
	3.08			3.42	
8	2.20	0.04	21	2.54	0.10
	2.16			2.44	
9	1.05	0.04	22	2.68	0.00
	1.09			2.68	
10	3.18	0.00	23	2.49	0.09
	3.18			2.40	
11	3.38	0.31	24	0.84	0.05
	3.07			0.82	
12	1.21	0.11		0.87	
	1.33			0.82	
13	1.96	0.09	25	0.76	
	2.05			0.87	0.11
				0.78	
14	2.09	0.31	26	1.54	0.17
	2.40			1.37	
	2.07	0.25	27	39.81	0.84
	1.82			40.65	

cushion, *O*. *H* is a perforated metal screen which serves as a seat for a white felt pad, *G*, about 2 mm. thick and a piece of filter paper, *F*, which is placed on top of the felt. *H* is soldered to the metal wire stand, *I*. The felt and the filter paper are cut with a cork borer to fit tightly into *J*. *E* is a perforated metal screen placed on the filter paper. The height of *I* is such that *E* is just below the top of *J*, when *I* rests on the bottom of the glass test tube.

*L* is a brass flange with 4 threaded holes, held in place by the collar, *K*, of tube *M*. The top flange, *B*, is countersunk to hold a neoprene washer, *C*. *A* is an air valve, of the type used on automobile tires, soldered into the center of the top flange.

OPERATING PROCEDURE. Approximately 1.8 cc. of the sample to be tested are weighed into the empty test tube *J*. The tube is placed in shell *M* and *I* is brought into the test tube, followed by the felt, the paper filter, finally plate *E*, and the top and bottom flanges are screwed together.

Thirty grams of Freon are charged into the test tube out of a Freon cylinder which is turned upside down and has a pressure hose connected to the outlet. This hose is closed by a self-closing valve such as are used by gasoline stations for putting air in tires. By pressing valve *A* against the valve in the hose, Freon is released into the test tube. *M* may be marked to indicate the height of liquid corresponding to 30 grams of Freon.

The test tube assembly is now placed in a beaker of water at 25° C. and kept there for about 5 minutes, turned up and down a few times to ensure proper solution, then centrifuged for 5 minutes in a clinical type of centrifuge. Now the tube is turned upside down and the Freon solution discharged by slowly pressing on *A*. Thirty grams of Freon are charged into the tube again and the procedure is repeated: bringing to 25° C., centrifuging, and discharging.

One or two additional washes with 20 grams of Freon may be required to make sure that all the soluble material is removed. No centrifuging is necessary for these washes. If the first wash liquor is colorless, the second wash is not necessary. Additional washes do not affect the final result.

After the last Freon wash has been discharged from the test tube, the tube is opened. Filter paper *F* is removed from the test tube, held with forceps, and any solid adhering to it is washed with benzene with the aid of a capillary pipet with rubber bulb into a tared crystallizing dish 75 mm. in diameter. This filter paper will usually be found to be clean. The felt is now removed from the test tube, held with the forceps, and washed with ben-



zene into the same crystallizing dish. *I* and the test tube itself are also washed out with benzene into the crystallizing dish. The Freon-insoluble solids are very readily soluble in benzene. The benzene solution is evaporated on a steam bath, the residue is placed for 20 minutes in a drying oven at about 90° to 100° C., and the dish is weighed:

$$\frac{\text{Weight of residue}}{\text{Weight of sample}} \times 100 = \% \text{ Freon-insoluble solids}$$

The ability to reproduce results is shown in Table I. The standard error of the mean of two duplicates of the first 26 samples

which range from 0.33 to 5.61 insoluble appears to be  $\approx 0.10$ . The duplicate determinations on sample 27 also show good agreement.

While this procedure was worked out for determining the solubility of pyrethrum extracts in Freon, it can be used for solubility determinations of other substances in solvents which are gases under normal pressures.

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## Simple Constant Reflux Take-Off for Distillation Systems

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IN LABORATORY fractional distillation it is often necessary to control the reflux ratio so that it is kept constant, regardless of variations in the distillation rate or empirical factors. This has been accomplished by Carter and Johnson (2) with a magnetically moved funnel, by Bruun (1) with a two-way valve and capillaries, and by Podbielniak (3) with an automatic valve seated in the take-off tube. All these devices require a degree of skill too great for fabrication by an amateur glass blower, and various mechanical difficulties have been experienced in their operation, due to sticking of moving parts or plugging of capillaries. An easily constructed take-off device of the intermittent type which operates smoothly is described in this paper.

#### CONSTRUCTION AND OPERATION

The take-off is designed for use with any type of total condensing head in which the entire condensate stream passes a point on its return to the column (see Figure 1). The receiver is maintained under a slight pressure sufficient to prevent the flow of liquid from the still head through the U-shaped tube into the receiver. A small steady stream of inert gas passing through the nonvolatile oil in the bubbler supplies this pressure. Periodically, the receiver is vented to the operating pressure of the still through an electrically operated valve controlled by a cycle timer. During this period the entire condensate flows by gravity to the receiver, by the mechanism shown in Figure 2.

While the receiver is under pressure, the liquid in the take-off capillary is maintained at levels *A, A'*. When the pressure is released, the liquid flows to levels *B, B'*, which are just at the overflow point. Consequently, all reflux during the "on" period enters the take-off at *A* and an equal amount of liquid overflows at *B'* into the receiver. At the end of the "on" interval (generally from 2 to 5 seconds), the valve closes and restoration of the receiver to pressure quickly interrupts the take-off stream. For

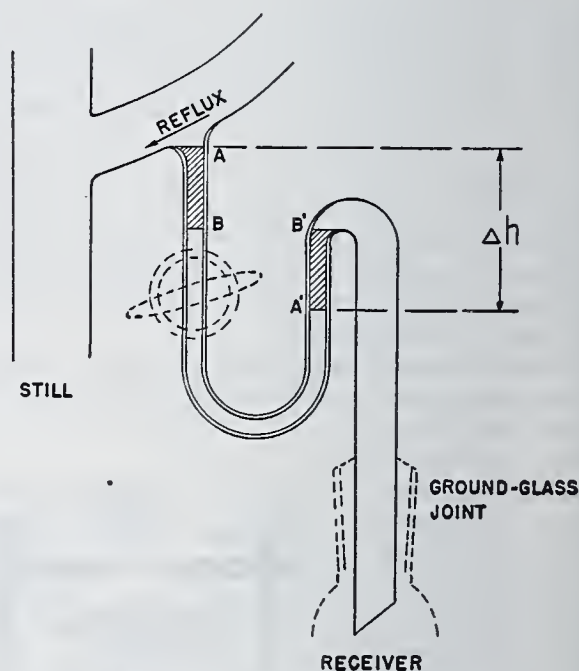


Figure 2. Detail of Take-Off Line

convenience in emptying the receiver, the take-off line is generally provided with a stopcock to prevent the flow during this period. However, if it is desired to keep the product from coming in contact with stopcock grease, and if low-boiling components which will vaporize in the take-off line at the outset of the distillation are absent, this stopcock and the one on the receiver may be omitted.

The dimensions of the take-off line depend mainly upon the throughput of the still, but are not critical. The diameter must be sufficient to accommodate the entire distillate during the take-off period. A convenient length for  $\Delta h$ , which indicates the pressure head that is maintained by the bubbler, is from 0.5 to 3 cm.

The following advantages have been noted:

Simplicity of construction, operation, and adjustment.

The take-off maintains a constant reflux ratio, irrespective of distillation rate, which is predetermined by simple adjustment of the intervals of an on-and-off timer.

Low cost. No delicate moving parts in the still head. Very low holdup.

Distillations may be conducted out of contact with anything save glass and an inert gas. The method is adaptable to distillation at other than atmospheric pressure. Several stills may be operated with the same control system.

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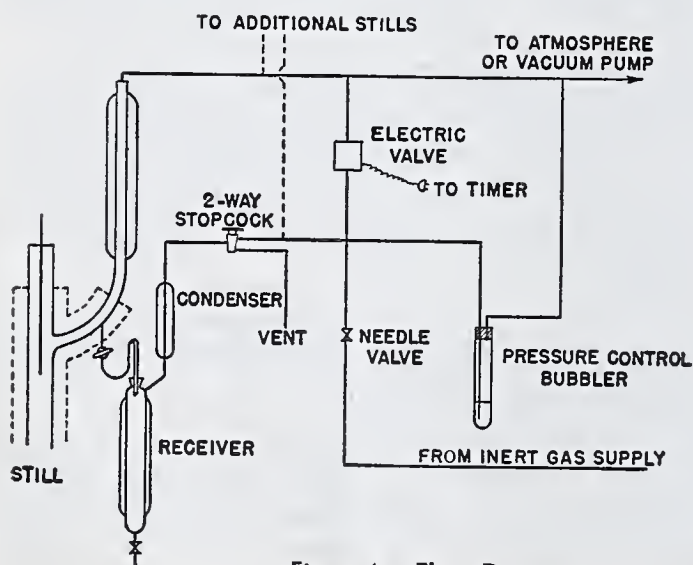


Figure 1. Flow Diagram



# A Spectrographic Method for Small Amounts of Calcium in Magnesium Metal

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A successful spectrographic procedure is described for the determination of low percentage calcium in magnesium metal using high-voltage spark excitation. Calcium is determined in amounts ranging from 0.0005 to 0.1%. Extrapolation of curves obtained by a calcium addition method gave results from which are established working curves for the direct spectrographic analysis of metallic magnesium.

NO SATISFACTORY chemical method for the estimation of as little as 0.01% of calcium in magnesium metal has been used by this laboratory. The method of Nikitina (5), in which magnesium metal sample is converted to metal chlorides and subsequently ignited to the respective metal oxides, is unreliable unless more than 0.1% of calcium is present.

The spectrographic method described herein involves measuring relative intensities of the spectral lines of calcium and magnesium. Subsequent extrapolation of relative intensity ratios obtained by adding known amounts of calcium to magnesium metal solutions makes possible the estimation of low calcium magnesium alloys.

Rendering magnesium salts calcium-free is most difficult. Magnesium nitrate recrystallized several times from nitric acid solution shows the greatest promise; nevertheless, a small amount of calcium still remains in the salt. Hughes (4) discusses several methods of purification of base compounds especially applicable to spectrographic standards. Duffendack and Wolfe and Cholak and Story (1) determine the amount of residual impurities present by observing differences between points on the curved portion of the analytical or working curve and the extrapolated straight-line portion at the lower percentage values of the material. Pierce and Nachtrieb (6) discuss the estimation of calcium elements and make comprehensive comparisons of methods of photometry used in most spectrographic analytical work.

Film calibration for the method herein described is made by using the logarithmic stepped sector in a manner similar to that described by Hasler (3).

## PROCEDURE

Three 1-gram samples of magnesium metal from the same refinery heat are dissolved in 20 ml. of 1 to 1 nitric acid and diluted to approximately 100 ml. with distilled water. Sufficient standard calcium sulfate solution is added to the first two samples to give the calcium contents 0.001 and 0.002% with respect to magnesium. No calcium is added to the third sample.

This same procedure is carried out for a series of five other refinery heats, making a total of eighteen samples in all. After thorough mixing, 3 drops of each sample are placed in slightly concave, specially purified, preburned graphite electrodes and held in an oven at 110° C. Each sample is subjected to an 8-pere direct current arc until completely vaporized. The exposures are made on an A.R.L.-Dietert grating spectrograph, using Eastman Kodak spectrum analysis film No. 1.

Ratios of relative intensity values of the calcium line 4226 Å. to the magnesium line 2936 Å. are evaluated, and these data are shown in Table I. Since the absolute intensity of the magnesium line 2936 Å. is reproducible within  $\pm 5\%$ , the relative intensity values are established using the gamma of the 4226 Å. region.

## DATA OBTAINED

The relative intensity ratios obtained from the samples with no additions of calcium and those with 0.001 and 0.002% additions

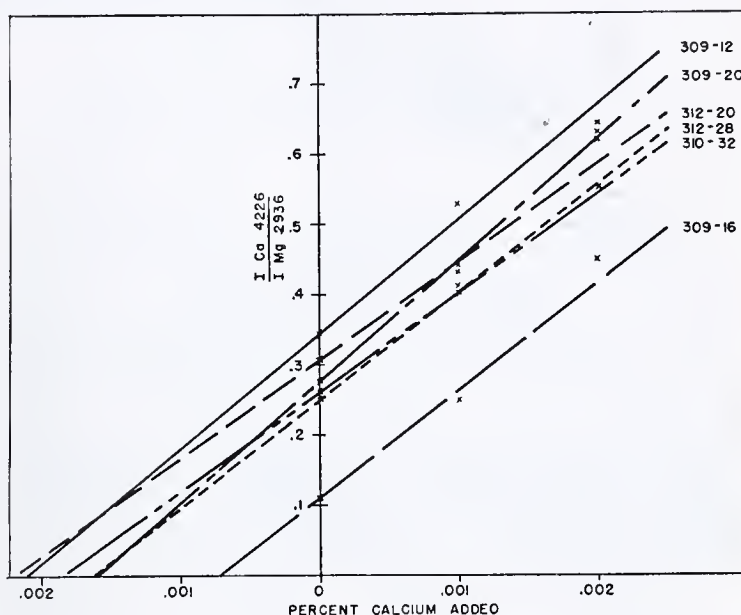


Figure 1. Extrapolation Curve

are plotted on the vertical axis, and the curve established by these three points is extrapolated to the horizontal axis. The amount of calcium present in each sample is obtained at the point where the extrapolated curve crosses the horizontal axis. This procedure is shown in Figures 1 and 2.

If the extrapolated values found, and the known additions plus the extrapolated values, are plotted on coordinate paper, using the intensity ratio as the ordinate and the per cent calcium as the abscissa, a curve will be obtained which originates at the zero point of both the horizontal and vertical axes.

A second series of samples containing calcium in amounts considerably higher than the first series is treated in a similar manner. However, only one addition of calcium, corresponding to 0.04%, is made. Ratios calculated for these samples are listed in Table II and plotted in Figure 2. A high-voltage spark is used to vaporize the sample in this series.

Table I. Relative Intensity Ratios of Calcium Line 4226 Å. to Magnesium Line 2936 Å.

(On nitrate solutions of magnesium samples with and without additions of known calcium)

Sample No.	0.00% Calcium Added	0.001% Calcium Added	0.002% Calcium Added
309-12	0.35	0.53	0.64
309-16	0.12	0.25	0.45
309-20	0.28	0.44	0.63
310-32	0.26	0.40	0.55
312-20	0.31	0.43	0.62
312-28	0.25	0.41	0.55

Table II. Relative Intensity Ratios of Calcium Line 3969 Å. to Magnesium Line 2779 Å.

(On nitrate solutions of magnesium samples with and without additions of known calcium)

Sample No.	0.00% Calcium Added	0.04% Calcium Added
2658	2.1	3.0
2660	2.0	2.85
2659	0.55	1.65
2661	1.49	2.32
2664	1.58	2.40



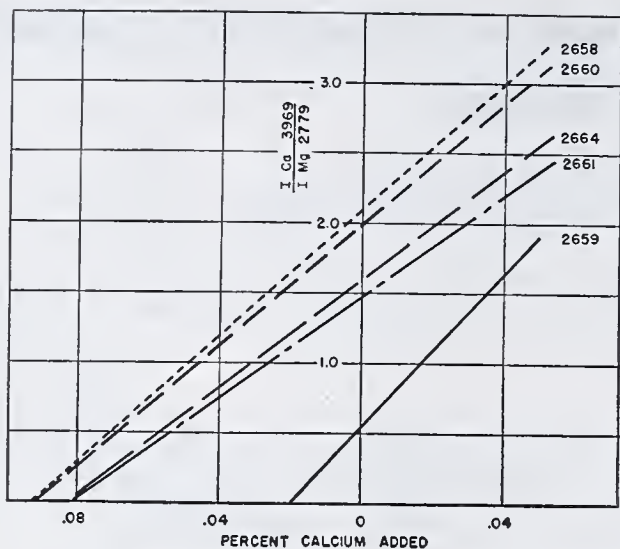


Figure 2. Extrapolation Curve

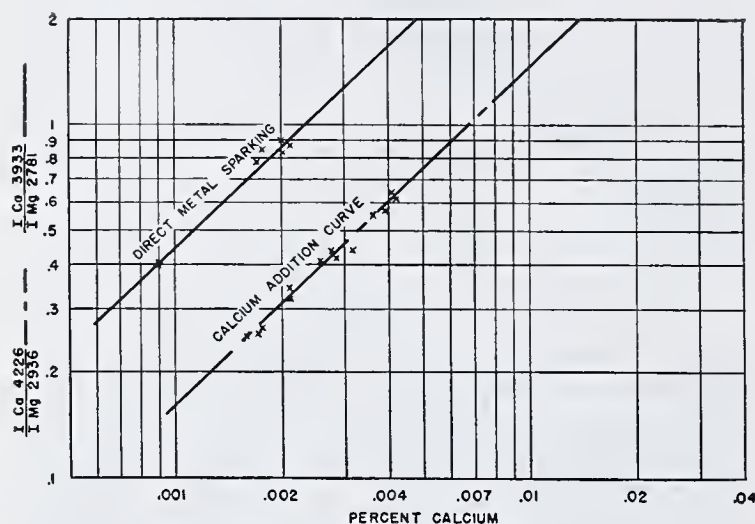


Figure 3. Working Curve

In order to make routine spectrographic estimations of calcium directly on metallic magnesium, the metal samples used to establish curves in Figures 1 and 2 are fastened in a lathe and faced smooth, then placed on a Petrey stand and subjected to a high-voltage spark, using a lower electrode of high purity graphite.

The working curve in Figure 3 is obtained by plotting the intensity ratio values of calcium 3933 Å. and magnesium 2781 Å. against the calcium percentages determined by extrapolation (Figure 1).

Satisfactory line densities are obtained on samples with higher calcium content by use of the logarithmic stepped sector. One eighth of the exposure can be secured by reading the calcium line in the fourth step of a logarithmic stepped sector having a ratio of 2 to 1 per step. The magnesium line used as a reference is read in the first step. Since the light is reduced by known amounts, the intensity ratio can be readily determined.

Table III compares results for calcium as determined by extrapolation in Figure 1 with results from sparking the metal samples directly. The results on samples of high calcium content (2658 to 2664, in Table III), are based on the direct metal working curve of Figure 3, using a logarithmic stepped sector procedure. Similar studies undertaken without the use of a stepped sector to establish data for direct calcium estimation of samples with higher calcium content give results agreeing very well with those in Table III.

Figure 4 shows a working curve for metal samples containing higher percentages of calcium, developed in the same manner as that shown in Figure 3. To a nitrate solution of 1 gram of the first three samples listed in Table III, sufficient calcium is added to

Table III. Calcium Found in Magnesium Samples

Sample No.	Method	
	By calcium addition	By direct metal sparking
	%	%
309-12	0.0021	0.0020
309-16	0.00075	0.0009
309-20	0.0016	0.0018
310-32	0.0019	0.0018
312-20	0.0022	0.0020
212-28	0.0016	0.0017
2658	0.093	0.108 <sup>a</sup>
2659	0.020	0.017 <sup>a</sup>
2660	0.092	0.112 <sup>a</sup>
2661	0.083	0.096 <sup>a</sup>
2664	0.083	0.096 <sup>a</sup>

<sup>a</sup> Logarithmic stepped sector values based on first six samples.

Table IV. Determination of High Percentage Calcium in Magnesium

(0.1% Ca added to samples listed in Table III)

Sample No.	Relative Intensity		Calcium Found %
	Ratio	Ca 3969 / Mg 2779	
309-12	2.10	0.1021	0.101
309-16	2.05	0.1008	0.099
309-20	2.05	0.1016	0.099

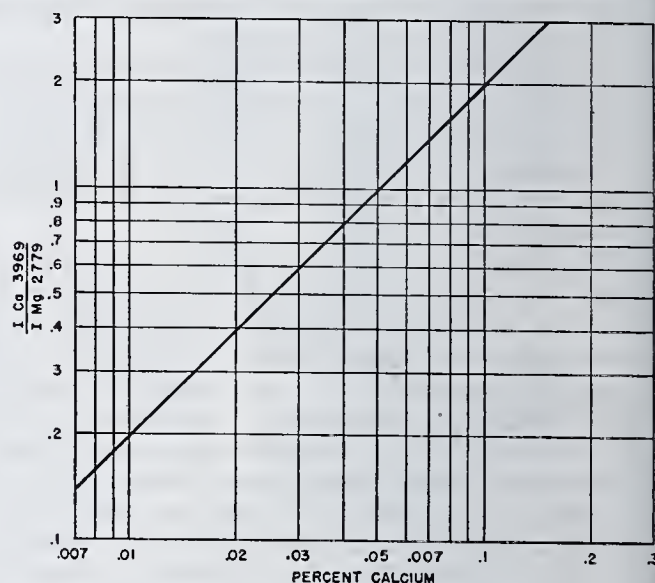


Figure 4. Working Curve

increase the percentage 0.1% with respect to the magnesium present. The percentages of calcium indicated when the ratio obtained by this procedure are referred to Figure 4 are listed in Table IV. The calcium thus found substantiates the data listed in Table II and plotted in Figure 4.

#### SUMMARY

A method is developed for the direct spectrographic estimation of low percentage calcium in magnesium metal samples. Calcium-free magnesium salts are difficult to obtain. Therefore known amounts of calcium sulfate solution are added to nitrate solutions of the magnesium samples to be analyzed. Subsequently intensity ratios of calcium and magnesium lines representing these samples are established, and percentages of calcium in the metal determined by extrapolation. A working curve for rapid routine analysis by sparking the metal directly is constructed.

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# Determination of Fluoride

## Detection and Determination of Acutely Toxic Quantities in Foods and Biological Material

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detailed procedure is given for a rapid, dependable, qualitative for detection of fluorides in foods and biological materials. This method will detect as little as 0.04% of fluorine as fluoride even in material with high carbohydrate content. A detailed procedure is also given for quantitative determination of fluorides in a variety of food products in the range 0.001% and up for certain carbohydrate-free foods and 0.01% and up for pure carbohydrates.

BECAUSE of the frequent occurrence of cases of accidental fluoride poisoning and the numerous requests this laboratory receives for complete toxicological examination of ingested materials and organs of deceased persons, it became necessary to develop methods for rapidly and positively determining the presence or absence of acutely toxic quantities of fluorides in samples containing a high percentage of organic material. The specific nature of the glass-etching test makes this test very desirable from a qualitative standpoint. In connection with samples containing a high percentage of organic material it is necessary to isolate the fluoride; this is most conveniently done by ashing a portion of the sample to be tested. It has been shown (6) that in the presence of much organic matter, such as sugar, up to 80% of the fluoride content of the sample may be lost, even in the presence of an alkaline fixative, such as lime suspension. Similar results have been experienced in this laboratory with samples of high sugar content, using the Brüning and East (4, 10) method of fixing fluorides by ashing. Accordingly, the method described below was developed as a systematic procedure for testing organic material for the presence of acutely toxic amounts of fluorides. In this method water-soluble organic material such as sugar is largely removed before ashing. The test is simple, rapid, and dependable and requires a minimum amount of material and reagents. It has never failed to reveal the presence of 10 mg. of fluorine as sodium fluoride in 25 grams (0.4%) of a variety of foods tested in this laboratory. The test works up unmistakably as a "frosted" circular spot about 2.5 cm. (1 inch) in diameter on a microscope slide.

### QUALITATIVE TEST FOR FLUORIDES IN FOODS AND BIOLOGICAL MATERIAL

Place 25 grams of the comminuted and well-mixed sample in a 100-ml. stoppered Erlenmeyer flask, add 75 ml. of water, and mix well until any sugar or other water-soluble organic matter is in solution. Add 2 ml. of 10% calcium chloride (anhydrous), 10 ml. of 10% copper sulfate pentahydrate solution, and make alkaline with sodium carbonate (10% solution), adding 15 ml. in excess. Mix well and allow to stand in a warm place for 1 hour. Cool to room temperature, and filter through a fluoride-free filter paper (Whatman No. 12, 18.5-cm. folded paper is convenient), using 5 ml. of water in rinsing flask. Transfer residue and paper to a nickel evaporating dish, dry carefully, and ash at 550° C. for 15 minutes. Crush ash and transfer to a platinum crucible. Place a clean, unetched microscope slide over the crucible and add enough concentrated sulfuric acid just to cover the ash. (Previously used slides by immersing in cleaning solution for 0.5 hour, rinsing with distilled water, and drying in an oven.) Heat over a small flame until sulfur trioxide fumes begin to appear. Continue the heating for 15 minutes. Cool, wash the slide well with distilled water, and dry in oven. If as much as 0.04% of fluorine as fluoride was present, the glass will be distinctly etched.

### QUANTITATIVE DETERMINATION OF FLUORIDES

The quantitative determination of small amounts of fluoride has been the subject of considerable study by numerous investigators for over 100 years.

Willard and Winter (15) introduced the distillation method for isolating fluorine, which involves constant-temperature volatilization of fluorine as hydrofluosilicic acid from aqueous solutions of sulfuric and perchloric acids. This procedure was an improvement on the volatilization methods used by Wöhler (16), Offermann (11) *et al.* Willard and Winter (15) also introduced the volumetric procedure of quantitative determination of fluorine based on titration with thorium nitrate, using a zirconium-alizarin mixture as indicator in alcoholic solution. Armstrong (2) titrated fluoride in an aqueous rather than alcoholic solution and Rowley and Churchill (12) applied the aqueous titration to the determination of quantities of 1 to 50 mg. of fluorine. Dahle *et al.* (8) studied a "back-titration" procedure as suggested by Allen (1). This procedure has been further studied by Clifford (5) and McClure (9) and was published in an up-to-date form by the referee on waters, brine, and salt in 1942 (3).

The subject of isolation of fluorine from organic material has received attention by various investigators. Preliminary distillation of fluorine from perchloric acid, in the presence of much organic material, may cause dangerous explosions.

Preliminary distillation from sulfuric acid was first suggested by Willard and Winter (15) and has been referred to by Wichmann and Dahle (13, 14). Direct ashing of the organic material followed by distillation of the ash has also received some attention. These efforts have been summarized by Clifford (6), but leave doubt as to the value of this method in general application to the determination of fluoride in foods and biological materials. Cox, Matuschak, Dixon, Dodds, and Walker (7) state that "in agreement with Armstrong, Dahle, and McClure the value of analysis for fluorine on materials which must be ashed is questionable".

Equipped with the foregoing more or less equivocal information, the authors attempted to develop a detailed, systematic, working method for determining acutely toxic quantities of fluorine in foods and biological material.

The resulting method was developed after numerous unsuccessful attempts to arrive at a judicious combination of detailed procedures which would isolate fluorine from various types of food and organic biological material (meat) in a form sufficiently free from interfering substances to apply the back-titration method. At the outset it was realized that the titration procedure was sufficiently sensitive and precise to work with small samples.

To avoid loss of fluorine by direct-ashing procedures because of presence of organic material, a preliminary distillation over sulfuric acid was made. Results showed that impurities in the distillate interfered grossly with titration of fluorine. An unsuccessful attempt was made to produce a pure distillate by oxidizing the volatile organic impurities in the first distillate, concentrating in an alkaline medium, and redistilling. Inasmuch as the organic material in the first distillate is low, experiments were carried out to determine optimum conditions for their removal by quick-ashing the first distillate after evaporating to dryness with fixative agents.

These experiments resulted in the present procedure. Since samples containing a high percentage of sugar or starch produced excessive foaming in the preliminary distillation with sulfuric



acid, a procedure was developed largely to destroy this material with potassium permanganate.

The authors feel that the workability of the back-titration method, as rewritten here, has been enhanced by use of stirring rods in the Nessler tubes. They believe that the use of a Nessler tube rack with white glass reflector and a large fluorescent titrating lamp contributes to the accuracy of the titration. Their experience shows that the titration is excellent for accurate measurement of amounts of fluorine in aliquots containing from 0.01 to 0.05 mg. after isolation of the element from interfering ions. Numerous practical details are included in both the isolation and titration procedures because it is felt that their observance is essential to the success of the method.

### PROCEDURE

The sample, consisting largely of organic material, is subjected to a Willard-Winter distillation using sulfuric acid, followed by evaporation of the distillate with sodium carbonate-lime water fixative and ashing for a short time in a nickel dish. The ash obtained is redistilled with perchloric acid and silver sulfate, thus providing a sufficiently pure aqueous solution of fluoride ion for accurate measurement by the back-titration procedure.

**REAGENTS.** Sodium fluoride solution. Dissolve 2.22 grams of sodium fluoride (purity at least 98%) in 1 liter of water (this solution contains 1 mg. of fluorine per ml.).

Standard sodium fluoride solution. Dilute 10 ml. of stock sodium fluoride solution to 1 liter (1 ml. = 0.01 mg. of fluorine).

Thorium nitrate solution. Dissolve 0.25 gram of thorium nitrate dodecahydrate in 1 liter of water.

Alizarin red indicator. Make a 0.01% water solution of sodium alizarin sulfonate.

Hydrochloric acid, (1 + 249) (0.05 N). Dilute 4 ml. of hydrochloric acid to 1 liter.

Sodium hydroxide solution, 0.05 N.

Potassium permanganate solution, 5%.

**APPARATUS.** Claissen flask, capacity 125 ml.

Nessler tubes, two or more long-form 50-ml. tubes matched for length, diameter, and optical similarity.

Stirring rods. Out of small-diameter glass rod make stirring rods with glass rings at bottom parallel to bottom of tube and of small enough diameter to fit into the Nessler tubes. The rods should be 5 to 7.5 cm. (2 to 3 inches) longer than the tubes, with the upper end bent at an angle, so that contents of the tube can be observed while stirring with an up and down motion.

**ISOLATION OF FLUORINE.** Transfer 10 grams of the comminuted and well-mixed sample into the Claissen flask (rinsed with boiling 10% sodium hydroxide to eliminate all traces of gelatinous silica), which has been set up for a Willard and Winter (15) distillation.

**Willard and Winter Setup.** Place the flask on an asbestos mat with an opening large enough so that about one third of the bottom of the flask will be exposed to the flame. Close the main neck with a two-hole rubber stopper through which pass a thermometer and a capillary glass tube, both extending within 2 to 3 mm. of the bottom of the flask. Connect a small glass funnel with a stopcock to the capillary tube, so that a controlled flow of water may be added during the distillation. Provide a solid rubber stopper for the side neck and connect the flask with a water-cooled condenser fitted with an adapter suitable for collecting the distillate in a 250-ml. Erlenmeyer flask.

Add 50 ml. of sulfuric acid (1 + 1) and several glass beads and distill until the temperature reaches 130° C. (use face shield during distillation). (The amount of water in the flask is not critical, so long as there is enough to cause the mixture to boil under 110° C. at the beginning.) Continue the distillation, holding the temperature between 130° and 140° C. by adding water through the capillary tube until 150 ml. of distillate have been collected in that temperature range. Drain condenser water and continue the distillation until steam is evolved from the end of the condenser.

Combine all distillate and transfer to a platinum or nickel evaporating dish. Neutralize with solid sodium carbonate and add 2 grams in excess. Add 10 ml. of lime water and evaporate to dryness. Ash for 5 minutes at 550° C. Cool and completely transfer ash to a Claissen flask with water, rinsing dish with 25 ml. of 60% perchloric acid. (This operation should be carried out carefully. Add the acid through a funnel inserted into the side neck of the flask which has been set up for distillation.) Add ca. 0.5 gram of pure, solid silver sulfate (preventing distillation of hydrochloric acid from salt in the sample, thus lowering acidity of the distillate; first suggested by McClure, 9) and re-

distill as before, but hold the temperature of the second distillation at 135° ± 2° C. after that temperature is reached.

Make up final distillate to a suitable volume—e.g., 500 ml. in a graduated flask and take a suitable aliquot for fluorine termination as follows:

**DETERMINATION OF ISOLATED FLUORINE.** Prepare one standard and one or more sample tubes as follows:

**Standard Tube.** Place 35 to 40 ml. of water in one of Nessler tubes equipped with stirring rod. Add 1 ml. of alizarin red indicator and 2 ml. of 0.05 N hydrochloric acid.

**Sample Tube.** Titrate an aliquot of the sample distillate with 0.05 N sodium hydroxide solution, using 1 ml. of indicator. Place a suitable aliquot (0 to 0.05 mg. of fluorine) in another Nessler tube equipped with stirring rod and make up to ca. 2 ml. with water. Add enough 0.05 N hydrochloric acid to total 2 ml., including acid in the sample aliquot, if any. Add 1 ml. of the indicator, mix, and add a measured amount of thorium nitrate solution from a microburet, dropwise, until a distinct faint pink color appears. Make up to 50-ml. mark with stirring rod resting in tube.

Add exactly the same volume of thorium solution to the standard tube as was added to the sample tube. Back-titrate from microburet with standard sodium fluoride solution, dropwise with stirring, until the color of the standard tube matches that of the sample tube. For final match adjust volume to that of sample tube.  $\text{Ml. of fluorine solution} \times 0.01 = \text{mg. of fluorine in sample aliquot}$ . Determine a reagent blank, using a sufficient amount of granulated sugar in place of the sample. Calculate fluorine content of original sample, correcting for reagent blank.

Table I. Fluorine in Various Types of Foods

Type of Sample	Wt. of Sample Grams	Sample Blank <sup>a</sup> Mg. F <sub>2</sub>	Sodium Fluoride Added Mg. F <sub>2</sub>	F <sub>2</sub> Recovered (Less Blank) Mg.	Recovery of F <sub>2</sub> %
Ground meat	10	0.10	10.00	9.80	98.0
Granulated sugar	1	0.10	10.00	9.40	94.0
Flour	1	0.06	10.00	9.88	98.8
Pea soup (concentrated)	10	0.07	10.00	9.70	97.0
Apple jelly	1	0.09	10.00	9.92	99.2
Canned spinach	10	0.06	10.00	9.92	99.2
Cola beverage	10	0.06	10.00	9.43	94.3
Powdered eggs	10	0.10	10.00	9.32	93.2

<sup>a</sup> Includes reagent blank and any fluoride present in original samples.

### NOTES ON THE METHOD

In case of excessive foaming or excessive charring of the sample during the distillation, such as would be caused by samples of high carbohydrate content, use less sample. The sensitivity of the method is such that as little as 1 gram of sample can be used provided the fluorine is evenly distributed in the sample.

Samples containing more than 50% of sugar or starch must be decomposed with potassium permanganate solution if as much as 1 gram of sample is used. Larger samples of other material may be advantageously treated with this reagent also if excessive foaming is encountered. The reagent is used as follows:

Warm sample with sulfuric acid in the Claissen flask to a temperature of 90° to 110° C. and add 5% potassium permanganate solution a few cubic centimeters at a time through a funnel in the side neck of the flask. Shake well until all the potassium permanganate has reacted, judged by disappearance of the pink color. Do not add an excess of potassium permanganate, as this may not only prove dangerous but also result in production of chlorine which would interfere in the final titration. Destroy as much organic material in this manner as is necessary to prevent excessive foaming during distillation. It is not necessary to eliminate organic matter completely at this stage. Of all the types of foodstuffs and meat tested, only high-sugar and high-starch foods required this treatment.

Results obtained by using the method on various types of food including meat, are shown in Table I.

An attempt was made to determine the source of the 0.06- to 0.10-mg. fluorine blank obtained by applying the method to the various foods. No titration error was observed when working with the standard thorium nitrate and sodium fluoride solution in the amount indicated in titration of the blanks.



taking each reagent in order, the following blanks were obtained by Willard-Winter distillation and back-titration procedure:

Reagents	Blank, Mg. of F
I Perchloric acid + $\text{Ag}_2\text{SO}_4$	0.00
II $\text{Na}_2\text{CO}_3$ + I	0.02
III Lime water (10 ml.) + I	0.01
IV $\text{H}_2\text{SO}_4$ + I, II, and III	0.05
V Granulated sugar (1 gram), 50 ml. of $\text{KMnO}_4$ + I, II, III, and IV	0.10

These results indicate, by difference, that the sulfuric acid liberated 0.02 mg. of fluorine to the blank. Since from 20 to 125 of potassium permanganate were used with the sugar, flour, soup, and apple jelly, the blanks obtained with these substances indicate that the permanganate may contribute as much as 0.05 mg. of fluorine, especially if it is assumed that pure granulated sugar contains no fluorine. The low blank obtained on each (which did not require use of potassium permanganate) sets this out. The high blanks obtained with ground meat and ordered eggs (0.10 mg. of fluorine), with which very little or no permanganate was used, indicate that these foods might contain p.p.m. of fluorine.

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## Quantitative Determination of High Molecular Weight Primary Aliphatic Amines

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A simple, rapid, and accurate method for quantitative determination of primary aliphatic amines containing 12 to 18 carbon atoms in the presence of their corresponding secondary amines is based upon the liberation of the primary amines by distillation. It can also be employed for the analysis of lower molecular weight primary amines and their salts in the absence of secondary amines.

The chemist working with fatty acid derivatives is frequently required to determine the amount of high molecular weight primary amine in a sample containing primary and secondary amines or their salts. While primary aliphatic amines can readily be determined quantitatively by titration with standard hydrochloric acid solution using methyl red indicator, analysis is complicated by the presence of secondary amines. Primary amines admixed with secondary amines have usually been separated by distillation, and then titrated with standard acid. For the high molecular weight amines, this procedure necessitates the use of vacuum distillation equipment, preferably a high-vacuum still with a minimum holdup, and consequently a relatively large amount of sample is required for the analysis. In addition, this procedure requires several hours for each determination. In view of the present expansion of commercial production and development of industrial uses for aliphatic amines, a simplified and accurate method for their analysis is desirable.

Such a method was mentioned in a recent paper (1) from this laboratory, discussing work in which it was necessary to determine the amount of amine and chloride ion in electrolyzed solutions of amine salts containing silver ions. Analysis for chloride in the presence of high molecular weight amines is complicated by the formation of an unfilterable colloidal heavy metal complex. Conductometric titrations are unsuccessful, owing to the fact that no sharp breaks are obtained when the conductivity of the solutions is plotted against the concentration of electrolyte. The higher amine salts of sulfamic acid were found to be sufficiently insoluble in water to indicate their quantitative precipitation. These salts are, however, soluble

in the presence of the alkali metal or alkaline earth ions which are necessarily introduced in the course of a gravimetric or conductometric analysis.

The procedure which was adopted for the analysis of amine hydrochloride solutions is essentially a modification of the Kjeldahl nitrogen determination. Instead of digestion of the organic nitrogen compound with the subsequent liberation of ammonia, the primary amine is liberated by an alkali and distilled directly into an acid solution. Further investigation of the method has demonstrated its applicability to all the common salts of the primary amines as well as to mixtures of high molecular weight primary and secondary amines and their salts.

#### METHOD AND DISCUSSION

**PROCEDURE.** The apparatus consists of the usual Kjeldahl setup. A Kjeldahl flask (600- or 800-ml.) is fitted with a connecting bulb to a straight condenser whose lower end is extended by a delivery tube into a receiver. Any type of modified Kjeldahl connecting bulb may be used.

To analyze for primary amine, a weighed portion of amine salt, or a known amount of amine salt solution, is placed in the Kjeldahl flask, the amine is liberated from its salt by addition of an excess (10% or more) of sodium (or potassium) hydroxide, and 200 to 400 ml. of water are added. The amine is then distilled into a known amount of standard hydrochloric acid solution. The amount of primary amine in the original sample can be determined by titrating the excess hydrochloric acid in the receiver with standard carbonate-free alkali, using methyl red indicator. When analyzing the higher amines, it is preferable to add a small amount of neutral ethanol to the acid in the receiver to dissolve the amine salt formed. The results of a number of determinations by this method are listed in Table I. The use of highly purified amine salts enabled accurate calculation of the amount of primary amine in the sample.

Table I shows the relative amounts of sample which can be analyzed conveniently. The values for the lower amines correspond to about 0.1 to 0.6-gram samples, and for the higher amines to 0.1 to 0.2-gram samples. These amounts of the lower amines distill in less than 30 minutes. Because of the lower vapor pressures of the higher amines (2), it is convenient to



Table I. Analyses of Amine Salts

Amine Salt	Amine Calculated Mole	HCl <sup>a</sup> Ml.	NaOH <sup>b</sup> Ml.	Amine Found Mole	Error %
C <sub>8</sub> H <sub>17</sub> NH <sub>2</sub> .HCl	0.002802	47.58	14.47	0.002800	-0.07
	0.00480	75.40	19.75	0.00480	0.00
C <sub>10</sub> H <sub>21</sub> NH <sub>2</sub> .HCl	0.00337	61.38	20.77	0.00337	0.00
C <sub>12</sub> H <sub>25</sub> NH <sub>2</sub> .HCl	0.001144	38.88	21.86	0.001144	0.00
	0.001144	36.22	19.69	0.001144	0.00
	0.003130	49.60	13.27	0.003125	-0.16
	0.003630	58.07	15.81	0.003628	-0.06
C <sub>15</sub> H <sub>33</sub> NH <sub>2</sub> .HCl	0.001058	32.20	17.16	0.001055	-0.28
	0.001175	30.71	15.77	0.001174	-0.09
	0.001175	34.18	17.72	0.001175	-0.00
C <sub>18</sub> H <sub>37</sub> NH <sub>2</sub> .HCl	0.000870	30.21	17.17	0.000869	-0.12
	0.000870	32.16	18.77	0.000868	-0.23
C <sub>18</sub> H <sub>37</sub> NH <sub>2</sub> .HCl + Na <sub>2</sub> SO <sub>4</sub>	0.000454	30.97	21.42	0.000453	-0.22
	0.000580	30.84	20.21	0.000580	-0.00
(C <sub>8</sub> H <sub>17</sub> ) <sub>2</sub> NH.HCl	0.001272	38.18	20.16	0.001270	-0.13
(C <sub>12</sub> H <sub>25</sub> ) <sub>2</sub> NH.HCl	0.000770	30.72	25.40	None	....
C <sub>18</sub> H <sub>37</sub> NH <sub>2</sub> .HCl + (C <sub>18</sub> H <sub>37</sub> ) <sub>2</sub> NH.-HCl + Na <sub>2</sub> SO <sub>4</sub>	0.000522 primary + 0.00022 secondary	34.82	23.99	0.000522	0.00

<sup>a</sup> 0.0935 N standardized with diphenylguanidine (4).<sup>b</sup> 0.1140 N prepared carbonate-free (3).

Table II. Distillation of Amines

Amine	Sample Gram	Mole	Time of Distillation Min.
Octylamine	0.1-0.5	0.0008-0.004	5-15
Dodecylamine	0.1-0.6	0.0005-0.003	10-30
			Hours
Octadecylamine			
Alkali only	0.1-0.2	0.0003-0.0006	1-1.5
Alkali + Na <sub>2</sub> SO <sub>4</sub>	0.1-0.2	0.0003-0.0006	0.75-1
Dioctylamine	0.1-0.3	0.0004-0.001	0.75-1.5
Didodecylamine (alkali only and alkali + Na <sub>2</sub> SO <sub>4</sub> )	None in over 1 hour		

use smaller samples for the sake of rapidity at the expense of greater accuracy. For the analysis of hexadecyl- and octadecylamines 30 to 50 grams of sodium sulfate may be added to the samples. This raises the boiling point of the system and materially reduces the time required to distill the primary amines. It can be seen from Table I that the addition of sodium sulfate does not increase the volatility of the corresponding secondary amines to the extent that they interfere with the analysis of the primary amines. Table II shows the time required for distillation of practical amounts of representative aliphatic amines.

**APPLICABILITY.** This procedure permits quantitative determination of aliphatic primary amines, containing from 12 to 18 carbon atoms, inclusive, in the presence of their correspond-

ing secondary amines. Salts of mixtures can also be analyzed by this method. When secondary amines are not present, the method may be employed for the determination of primary aliphatic amines and their salts up to and including octadecylamine. The quantitative removal of the amine component allows determination of the anion in the residual solution by usual procedures. Since the presence of the amine frequently complicates the accurate determination of the anion, this procedure removes one of the difficulties encountered in the analysis of high molecular weight amine salts.

When the method is applied to the analysis of a mixture of primary and secondary amines, it is entirely dependent upon the differences in their respective vapor pressures at the boiling point of water. The limitations as to chain length have been designated above.

**INTERFERING SUBSTANCES.** The most frequently encountered interfering substance in commercial amines is ammonia, which can be removed readily by a preliminary heating of the alkaline sample under reduced pressure. At 50° C. and 20-mm. pressure the amount of the higher primary amines lost by vaporization is within the experimental accuracy of the analysis (2). Other volatile organic bases are not generally encountered in such mixtures. None of the other common impurities of the commercial aliphatic amines interferes with the method: nitro compounds distill simultaneously with the amine, but does not react with the acid in the receiver; free fatty acid is converted to soap and prevented from hydrolyzing by the excess alkali; amide does not effect the analysis.

**ACCURACY.** Table I shows the accuracy which can be obtained by this method if reasonable care is exercised. Smaller samples than those suggested can be analyzed accurately by using more dilute standard acid and alkali solutions. Since the composition of commercial amines cannot be determined accurately by any other method, no direct check can be made upon the accuracy of the analyses of these compounds by this method. However, the results agree within 1 to 2% with separations of these compounds by distillation.

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## Rapid Determination of Zinc in Magnesium Alloys

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**METHODS** ordinarily used in the quantitative analysis of zinc in magnesium alloys employ preliminary separations and subsequent determination of the zinc with potassium ferrocyanide (2, 3) or as zinc oxide adapted from an aluminum alloy method (1, 8). Methods employing electroplating in a sodium hydroxide medium after preliminary separations (6, 9), and an iodometric titration procedure have appeared (4).

A rapid and direct means of obtaining results with no preliminary separations was attempted in this laboratory and the electrodeposition of the zinc in ammoniacal solutions containing ammonium chloride (5, 7, 10) was found to give results well in agreement with the classical methods. Weak organic acid solutions were also used for the deposition of the zinc by the authors, but the method is more time-consuming and offers no advantage over the electroplating in ammonium hydroxide.

Sufficient ammonium chloride is introduced to prevent the precipitation of magnesium hydroxide, and tartaric acid also is added to keep the aluminum present in solution. The total time needed for a single determination is about 25 minutes. Sources of error due to manipulation are removed, as the determination is completed in one beaker.

## PROCEDURE

Dissolve a 0.5-gram sample in a 200-ml. electrolytic beaker by adding 25 ml. of dilute sulfuric acid (1 to 14). Add 2 ml. of tartaric acid solution (25%) and 17 to 20 grams of ammonium chloride, dilute to 100 ml., and stir to dissolve the salts. Make the solution barely ammoniacal to rosolic acid (or any neutral indicator) and add 4.0 ml. of ammonium hydroxide (0.90 N) with stirring. Electrolyze for 20 minutes at 2 amperes, with a gentle stream of air agitating the solution. (Turn the current on before the beaker is placed in contact with the electrodes to avoid an



**Table I. Comparison of Electrolytic Method with Standard Methods**

Alloy	Alloying Elements	Zinc (Electrolysis) %	Zinc (Ferrocyanide) %	Zinc (Gravimetrically) %	Deviation %
65	3% Zn, 6% Al, 0.2% Mn	3.07	3.05	..	+0.02
65	.....	3.04	..	3.00	+0.04
65	.....	3.20	3.22	..	-0.02
65	.....	3.02	..	3.01	+0.01
65	.....	3.30	..	3.18	+0.12
60	2% Zn, 9% Al, 0.2% Mn	2.34	2.44	2.30	....
60	.....	2.30	2.32	2.32	....
7-S	1% Zn, 6% Al, 0.5% Mn	1.05	..	1.10	-0.05

**Table II. Precision of Electrolytic Method**

Sample	Zinc %	Deviation %	Sample	Zinc %	Deviation %
1	3.20	0.03	2	2.50	0.02
1	3.24	0.01	2	2.48	0.00
1	3.20	0.03	2	2.52	0.04
1	3.22	0.01	2	2.52	0.04
1	3.24	0.01	2	2.44	0.04
1	3.26	0.03	2	2.42	0.06
			2	2.48	0.00
			2	2.46	0.02
Av.	3.23	0.02	Av.	2.48	0.03

sible solvent action of ammonium hydroxide on the copper or nickel of the cathodes.) Wash by rapidly lowering the electrolyte and replacing with a beaker of distilled water. Repeat the operation using a second beaker of water. Immerse the electrode in neutral alcohol and place in an oven at 100° C. until alcohol has completely evaporated. Remove immediately from the oven to prevent oxidation of the zinc plate, cool to room temperature, and weigh.

The zinc-plated electrode is best kept in a desiccator if it is not weighed as soon as cool. Platinum electrodes which have a bit unoxidized copper plate or nickel electrodes are used as cathodes and are tared before using. A platinum wire spiral as the anode. Gauze cathodes 4.4 cm. (1.75 inch) in diameter by 5 cm. (2 inches) in length are used. The nickel electrode may be stripped of zinc by immersing in dilute sulfuric acid (p. 15).

appreciable amounts of lead, tin, cadmium, copper, or silver present, a hydrogen sulfide separation is necessary. In this case use small flasks for dissolving the sample, dilute to approximately 70 ml., and pass a rapid stream of hydrogen sulfide through the solution for 10 minutes. Warm on a steam bath a few minutes to coagulate the precipitated sulfides. Cool in a stream of running water and filter through a close paper, catch the filtrate in a 200-ml. electrolytic beaker; wash with acidified hydrogen sulfide water. Boil the filtrate until free of hydrogen sulfide and cool to room temperature. Now proceed as in routine method.

## RESULTS

The purity of the zinc deposit and the completeness of the electrodeposition were determined on the pure salt solutions. The zinc plate showed the presence of a trace (less than 0.1 mg.) of manganese and a trace of iron. The electrolyzed solution was analyzed for zinc colorimetrically by means of dithizone and 0.1 or less was found to be present. Lead, tin, silver, and cadmium if present also deposit, causing high results and must therefore be removed prior to electrolysis.

Regularly encountered types of magnesium alloys were analyzed by the three procedures discussed; the comparative results are shown in Table I. The reliability of the method is shown by results obtained by two different analysts in Table II.

## DISCUSSION

The accuracy of the direct electrodeposition of zinc in magnesium alloys compares favorably with that of the zinc oxide or ferrocyanide procedures and the electrolytic method is much better. The deposits are sufficiently pure and quantitative for routine analytical purposes, as shown by tests of the zinc plate from the electrolyzed solution which was composed of pure salts in known amounts.

The zinc deposit is bright, metallic, and adherent, but is easily oxidized during electrodeposition if the copper-plated electrode is oxidized or during the drying period if too prolonged. Nickel electrodes give slightly more consistent results and avoid the necessity of obtaining good copper deposits on the platinum electrodes. The current is best turned on before the electrodes are in contact with the electrolytic solution, to avoid any tendency of the ammoniacal solution to attack the copper plate or nickel which will be deposited again but not always bright and free of oxides.

Elements interfering with the direct deposition of zinc are seldom encountered in appreciable amounts. Interference of copper is prevented by selective solution, so that the copper remains undissolved. The residue need not be filtered for routine work unless it is large. Silver, lead, tin, and cadmium are not present ordinarily. The first three elements are almost completely removed from solution through displacement by undissolved magnesium when they are present in amounts as small as 0.5 mg. The insoluble residue is removed by filtration. However, when any or all of these elements are present in larger quantities or as alloying elements, a hydrogen sulfide separation is necessary. The addition of hydrogen sulfide to several commercial alloys tested gave no visible precipitate. Iron also will interfere if present, but, fortunately, it is present only as a trace in magnesium alloys.

## SUMMARY

The rapid routine determination of zinc in magnesium alloys is accomplished by the direct electrolysis of the samples, after selective solution, in ammoniacal medium. The elements interfering with the deposition are seldom encountered in magnesium alloys and if present at all are ordinarily in trace quantities and need no separation. Significant amounts of elements which are electrodeposited in acid solution must be separated. A hydrogen sulfide precipitation is proposed.

Deposition is rapid and quantitative in 20 minutes and the entire determination need require but 25 minutes. The electrolytic method compares favorably with the accepted procedure.

**Table III. Precision Employing Pure Salts**

Amount Added					Found
Zn	Al	Mn	Mg	Pb	
Mg.	Mg.	Mg.	Mg.	Mg.	Mg.
10.0	..	..	..	..	10.1
10.0	65.0	2.5	900	..	9.9
10.0	65.0	2.5	900	..	10.1
10.0	65.0	2.5	900	..	10.0
10.0	..	..	..	2.0	11.6

## ACKNOWLEDGMENT

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# Precision and Accuracy of Colorimetric Procedures as Analytical Control Methods

## Determination of Silica

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A colorimetric procedure for the rapid determination of small amounts of silica, involving the reduction of the silicomolybdate complex to the intense molybdenum blue, has been developed to meet the special requirements of analyzing leach liquors. The quantities and concentrations of reagents specified are considered to be best for color stability, sensitivity, and speed of color development. Factors influencing color intensities have been investigated, and techniques for a precision and accuracy of a control character are described. Precision and accuracy studies on data from ordinary routine analyses of leach liquors have been made. The average precision measured by the average deviation of single results from the mean is of the order of  $\pm 1\%$ , while the over-all accuracy is of the order of  $\pm 1\%$ .

A PREVIOUS article (12) outlined an experimental procedure for the colorimetric determination of aluminum and described the requisite techniques for precision and accuracy of a control character.

Further studies on methods of analytical control have resulted in the development of a procedure for the colorimetric determination of silica. This is a modification of previously published methods, and the investigation evaluates the factors that influence precision and accuracy as employed in routine analyses. Statistical reasoning based on the standard deviation is applied to the acquired data (1).

The usual procedure for the colorimetric determination of soluble silica depends on either the formation of the yellow silicomolybdate color produced when ammonium molybdate reacts in an acid media with the soluble silica (4, 10, 13) or the reduction of the silicomolybdate complex to the intense molybdenum blue color (3, 7).

Edwards (5), noting the difficulties inherent in the determination of silica in calcined alumina, suggests the use of the silicomolybdate as a means of rapid and accurate estimations of small amounts of silica.

The procedure employing the formation of the molybdenum blue color was selected for the rapid analysis of small amounts of silica in leach liquors in pilot-plant operations, a leaching step in the lime-soda process for the extraction of alumina from siliceous bauxites and clays. It is believed that an extended sensitivity of the reaction will be reflected in more accurate results. The interferences cited by Knudson, Juday, and Meloche (10) were not present in the sample.

Kahler (7) develops the silicomolybdate complex by the use of hydrochloric acid, considering a pH range of 2.4 to 2.7 as optimum prior to reduction, and reduces the yellow complex with sodium sulfite. However, this method is unsatisfactory for the analysis of leach liquors, since the pH (6.8) of the final solution closely approaches optimum conditions for precipitation of aluminum hydroxide. To minimize color progression and prevent precipitation of aluminum hydroxide, an organic reducing agent (8, 9) has been investigated and found to be applicable.

The recommended quantities and concentrations of reagents in the following procedure are considered to be best for color stability, sensitivity, and speed of color development.

### ANALYTICAL PROCEDURE

**REAGENTS.** Standard Silica Solution. Dissolve 47.32 grams of sodium metasilicate ( $\text{Na}_2\text{SiO}_3 \cdot 9\text{H}_2\text{O}$ ) in 1 liter of water (1 ml. = 10 mg. of silica), filter, and standardize gravimetrically (6). Store in rubber bottle (14).

**Working Standard.** Dilute 2 ml. of standard to 1000 ml. (1 ml. = 0.02 mg. of silica) and store in a rubber bottle.

**Reducing Solution.** Dissolve 2 grams of 1,2,4-aminonaphtholsulfonic acid, 120 grams of sodium metabisulfite, and 100 grams of sodium sulfite in water and dilute to 1 liter. Store solution in a dark bottle in a cool place.

**Ammonium molybdate,** 5% solution in water. **Hydrochloric acid,** 0.5 N.

**Indicator,** 2,4-dinitrophenol or 2,6-dinitrophenol. Saturated solution in water.

**Ammonia Gas.** Bubble clean air through ammonium hydroxide.

**PROCEDURE.** Discharge an aliquot of the acidified solution containing 0.02 to 0.10 mg. of silica, into a 25-ml. calibrated blood-sugar test tube (12) by means of a pipet. Add 2 or 3 drops of indicator, and bubble ammonia gas into the solution to the yellow end point. Add 5 ml. of 0.5 N hydrochloric acid and enough distilled water to bring the meniscus to the 25-ml. mark and mix the contents of the tube thoroughly. Add 2 ml. ammonium molybdate by means of a pipet, mix thoroughly and permit the solution to stand 5 minutes. Add 3 ml. of reducing solution by means of a pipet, mix again, and determine the color absorption with the Klett-Summerson photoelectric colorimeter equipped with a filter having a transmission range of 640 to 700 m $\mu$ . It is absolutely essential to run a blank with the reagents, the correction being applied to the colorimeter reading.

A standardization curve is prepared from a series of points of known silica content. Since the curve is linear throughout the concentration range used in this study (0.01 to 0.28 mg. of silica), it will be found convenient to calculate a conversion factor. A quantity, in triplicate, of the working standard, 2 or 3 ml. discharged into blood-sugar test tubes and the color is produced in the usual manner. After the blank correction is made, the scale division is evaluated in terms of milligrams of silica.

The conversion factor used in this investigation, 0.000311 mg. of silica per scale division, should not be selected as an arbitrary constant since the value of the factor would not necessarily hold for other instruments. A factor must be regarded as valid only under the conditions of calibration.

The product of a given scale reading and 0.002, an arbitrary selected proportionality factor (14), results in an approximate density reading where density =  $\log 1/\text{transmission}$ .

### FACTORS INFLUENCING RESULTS

In a study on the reproducibilities of blanks, the foregoing procedure was applied to several milliliters of slightly acidified distilled water. It has been found that the presence of impurities on walls of blood-sugar test tubes, supposedly chemically clean, has a pronounced effect upon the colorimeter reading. It is highly advisable to label new test tubes and use them specifically for colorimetric silica. Chromic-sulfuric acid mixture effectively cleans the new tubes; the use of alkaline cleaning mixture not only is unnecessary but is inadvisable, since etched surfaces induce irregular results. It is extremely important to make blank corrections in routine analyses, as impurities in test tubes and/or reagents can markedly affect the final reading.

The instability of the blue color has been cited (2, 10) as an objection to the use of reduced silicomolybdate in colorimetric silica. As shown in Table I, the color is stable for several hours. In this particular range of the colorimeter scale, the 2-unit increase in reading represents one scale division.



Table I. Stability of Molybdenum Blue

(Colorimeter readings on standard silica, uncorrected for blank)

Elapsed Time, Minutes	No. 1	No. 2	No. 3
0	206	208	208
15	206	206	208
30	206	208	208
45	208	208	210
60	208	208	210
90	210	210	210
120	210	208	210
165	210	210	210
195	210	210	210
255	212	212	212
315	212	212	214
Overnight	264	260	268

Table II. Effect of pH on Colorimeter Reading

Color (End of Titration), 2,6-Dinitrophenol	pH	Final volume	Colorimeter Reading (Corrected for Blank)	Error, %
Standard Soluble Silica: 0.0590 mg. of SiO <sub>2</sub>				
Very faint yellow	1.02	1.98	188	-0.5
Very light yellow	1.01	1.99	188	-0.5
Light yellow	1.02	1.99	188	-0.5
Yellow	1.04	2.02	189	0
Deep yellow	1.06	2.08	189	0
Deep yellow	1.21	2.39	185	-2.1
Standard Soluble Silica: 0.0590 mg. of SiO <sub>2</sub> and Ions <sup>a</sup>				
Very faint yellow	1.02	1.91	189	0
Faint yellow	1.07	1.98	188	-0.5
Light yellow	1.10	2.04	185	-2.1
Yellow	1.11	2.11	183	-3.2
Yellow	1.18	2.20	180	-4.7
Yellow	1.27	2.42	177	-6.3

1 ml. of standard = 0.01 mg. of SiO<sub>2</sub> + 10 mg. of Al<sub>2</sub>O<sub>3</sub> + 10 mg. of O (SiO<sub>2</sub>:Al<sub>2</sub>O<sub>3</sub> = 0.1%).

Since the pH in this analytical procedure is critical, it becomes necessary to adjust all samples to approximately the same hydrogen-ion concentration. In the presence of a suitable indicator the previously acidified aliquot is neutralized with aqueous ammonia, a technique designed to eliminate the introduction of measurable amounts of silica in the use of dilute ammonium hydroxide. Either 2,4-dinitrophenol (pH 2.6 to 4.0) or 2,6-dinitrophenol (pH 2.0 to 4.0, 11) may be used as the indicator, since the yellow color changes to colorless at about pH 4.0, thereby resulting in no complications from added color.

The effect of varying degrees of neutralization on standard soluble silica with and without the addition of aluminum and sodium ions was studied in terms of the resultant colorimeter reading. In Table II, six test tubes, corresponding to samples 1 to 6, each contained 3 ml. of working standard and each sample was treated with successively greater amounts of ammonia gas. It appears that the end point is not easily overstepped in the titration of the standard. Only sample 6 shows appreciable changes. In carrying out a titration, two-color changes usually are observed, first a light yellow and next a deep yellow. A satisfactory procedure is established by stopping the titration at the appearance of a faint yellow since, on rinsing the gas tube, the residual ammonia gas changes the color to a deep yellow.

The pH of these solutions was measured by means of a glass electrode and Beckman pH meter after dilution had brought the meniscus to the 25-ml. mark and after all ingredients had been added. The numerical value for pH, although not initially reflected in the colorimetric reading, shows a gradual change as the series is descended. Only No. 6, representing a definite excess of ammonia, shows significant changes. In ordinary laboratory practice, it is doubtful if titration would be carried to the extremes of Nos. 5 and 6.

The second set of test tubes in Table II, samples 1a to 6a, represents a study on the effect of varying degrees of neutralization of standard soluble silica in the presence of aluminum and sodium ions. Aluminum chloride hexahydrate and sodium hydroxide were added to the working standard in amounts calculated to contain 10 mg. per ml. each of aluminum trioxide and sodium oxide. Blank determinations with and without the ad-

dition of aluminum and sodium ions indicated the absence of soluble silica in these reagents.

The data in the second part of Table II indicate that the overstepping of end point of the standard in the presence of these ions results in decreased accuracy of a negative order. The intensity of yellow in tubes 1a and 2a was obtained by stopping the titration at the appearance of a white precipitate, aluminum hydroxide, with no evidence of yellow color, and flushing out the residual gas in the tube with a small amount of water. The formation of this precipitate probably results from localized neutralization; however, since the precipitate disappears on shaking the tube, its presence does not appreciably impair the value of the procedure. The samples in the remaining tubes were titrated to various shades of yellow before the residual ammonia gas was rinsed out. However, the Al<sub>2</sub>O<sub>3</sub>:SiO<sub>2</sub> ratio in this synthetic mixture is much higher than that usually encountered in routine analysis.

## PRECISION AND ACCURACY

The precision of the colorimetric method may be studied by applying the procedure to the analysis of leach liquors. Three typical leach liquors previously acidified and diluted (10 ml. in 250 ml.) were selected, and a 1-ml. portion of each sample was taken for analysis. The silica content was measured over a period of several days. Table III shows what might be expected in the way of precision for 10 determinations on each sample. The method of evaluating the factor of precision is that derived from consideration of results set up by A.S.T.M. (1). The accuracy of the method is determined by making a comparative study of standard soluble silica with and without the addition of aluminum and sodium ions (Table IV).

## DISCUSSION

In computing the  $\pm$  values for the results in Table III, a value for  $a$  was chosen such that, in 99 chances out of 100, one

Table III. Precision of Method under Routine Conditions

Test No.	Sample 1			Sample 2, Total SiO <sub>2</sub> Mg.	Sample 3, Total SiO <sub>2</sub> Mg.
	Total SiO <sub>2</sub> Mg.	$d$	$d^2 \times 10^8$		
1	0.0899	-0.0002	4	0.1240	0.1581
2	0.0899	-0.0002	4	0.1256	0.1550
3	0.0893	-0.0008	64	0.1240	0.1550
4	0.0915	+0.0014	196	0.1256	0.1550
5	0.0899	-0.0002	4	0.1240	0.1581
6	0.0899	-0.0002	4	0.1256	0.1581
7	0.0899	-0.0002	4	0.1240	0.1581
8	0.0899	-0.0002	4	0.1240	0.1550
9	0.0893	-0.0008	64	0.1225	0.1550
10	0.0915	+0.0014	196	0.1240	0.1550
Av. = $\bar{X}$ =	0.0901			= 0.1243	= 0.1562
$\Sigma d^2 =$	$544 \times 10^{-8}$			= $885 \times 10^{-8}$	= $2308 \times 10^{-8}$
$\sigma_{10} = \sqrt{\frac{\Sigma d^2}{10}} =$	0.0007			= 0.0009	= 0.0015
$\bar{X} \pm a\sigma =$	$0.0901 \pm 0.0008$			= $0.1243 \pm 0.0010$	= $0.1562 \pm 0.0016$

(P<sub>s</sub> = 0.99, 10 observations)Table IV. Accuracy of Method under Routine Conditions  
(Colorimetric silica)

Sample No.	SiO <sub>2</sub> :Al <sub>2</sub> O <sub>3</sub> %	Analysis, Total Silica Calculated Mg.	Found Mg.	Error %
1	0.1	0.0393	0.0392	+0.3
2	0.1	0.0590	0.0587	-0.6
3	0.1	0.0786	0.0778	-1.1
4	0.5	0.0590	0.0589	-0.2
5	1.0	0.0590	0.0590	0
6	1.0	0.0590	0.0590	0
7	1.0	0.0590	0.0596	+1.2
8	1.0	0.0590	0.0590	0
9	2.0	0.0590	0.0593	+0.8
10	3.0	0.0590	0.0593	+0.8
11	4.0	0.0590	0.0593	+0.8
12	5.0	0.0590	0.0593	+0.8



might expect the ranges bounded by the computed limits to include, of the universe sampled, the objective average,  $\bar{X}$ .

From a consideration of the data in Table IV, it is apparent that the over-all accuracy is of the order of 2%. Since the  $\text{SiO}_2:\text{Al}_2\text{O}_3$  ratio markedly affects the accuracy, it is believed that the ratios investigated in Table IV cover the ranges usually encountered in routine work. Repetition of 0.0590 mg. of silica per ml. in this study is explained by the fact that this concentration provides a reading in the most satisfactory portion of the scale for colorimetric measurement (200). For this study, 3 ml. of working standard were admixed with varying proportions of aluminum and sodium ions. In the range of 0.1% ( $\text{SiO}_2:\text{Al}_2\text{O}_3$ ), the results invariably are low, probably indicating that the end point had been overstepped. In the range of low silica to high alumina ratios, the color change in the titration procedure is not too abrupt. In this regard it has been found that 2,6-dinitrophenol, owing to depth of color, furnishes a more distinct end point than 2,4-dinitrophenol. However, in the range of 1.0 to 5.0%, no particular difficulty is experienced in observing color changes of the indicator.

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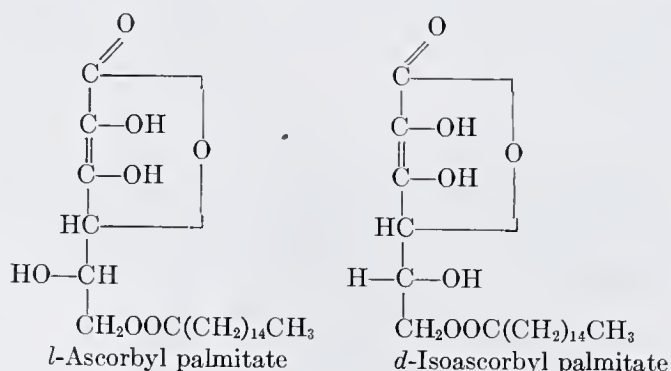
# Determination of Fatty Acid Monoesters of *l*-Ascorbic and *d*-Isoascorbic Acids in Fats and Oils

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The 2,6-dichlorophenolindophenol reagent in acetone can be successfully used for the determination of the fatty acid monoesters of *l*-ascorbic and *d*-isoascorbic acids in fat and oil substrates.

**M**ONOESTERS of *l*-ascorbic and *d*-isoascorbic acids have been prepared (4) by direct esterification of the ascorbic acids with fatty acids. According to the experimental evidence, the esterification probably takes place on the primary alcohol group of the ascorbic acids, as illustrated by the following formulas:



In previous work in this laboratory (2) it was found that these ascorbyl esters were effective antioxidants for fats and oils. (For convenience of expression, the fatty acid monoesters of *l*-ascorbic acid and *d*-isoascorbic acid are referred to in this paper as the ascorbyl esters. It has been suggested that these esters be named as derivatives of the ascorbic acids—for example, palmitoyl *l*-as-

corbic and palmitoyl *d*-isoascorbic acids.) In connection with an investigation of the antioxidant action of the ascorbyl esters, the results of which will be published later, it was essential to have an analytical method for determining ascorbyl esters in fat substrates.

Ascorbyl esters are somewhat soluble in fats and oils but are insoluble in water, whereas the ascorbic acids are insoluble in fats and oils but very soluble in water. Since the ene-diol group is unchanged when ascorbic acid is converted to the monoester (4), the methods for determining ascorbic acid which are dependent upon the reactivity of the ene-diol group should be applicable for the determination of the ascorbyl ester. One method that has been used for determining ascorbic acid involves titration with iodine solution (3). It is obvious, however, that this method would be unsuitable with fat and oil substrates, since these contain unsaturated groups, which absorb iodine. The most widely used method today is that originally suggested by Tillmans (5), in which the ascorbic acid in aqueous solution is titrated with 2,6-dichlorophenolindophenol. In order to use this method for ascorbyl esters in fat substrates, considerable modification was necessary, since these esters are insoluble in water. Various solvents (absolute ethyl alcohol, ethyl ether, diethyl ether, and acetone) were tried and acetone was found to be preferable.

#### STANDARDIZATION

Standardization of the indophenol solution is carried out as follows: A known amount of the sodium salt of 2,6-dichlorophenolindophenol is dissolved in dry acetone of AMERICAN CHEMICAL SOCIETY grade. A solution containing 0.25 gram of the indophenol salt in 1 liter of acetone may be used for deter-







# Further Studies of the Molybdenum Blue Reaction

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As a result of a spectrophotometric study of the determination of phosphorus, a modified A.O.A.C. procedure is presented which gives solutions of greater color stability and of equal color intensity. The work includes a determination of the effects of the following variables: reagent concentration, temperature of color development, order of addition of reagents, phosphorus concentration, and sixty diverse ions.

ONE of the most frequently used methods for the colorimetric determination of phosphates is the so-called molybdenum blue procedure, which depends upon the formation of molybdi-phosphoric acid, with subsequent reduction to a blue system of uncertain composition. As the blue color formed is unstable, many modifications have been suggested in the effort to get a more nearly stable system. Woods and Mellon (9) give a review of some past work, together with the results of a spectrophotometric study of some of the methods.

In a recent article Stoloff (8) proposed a modification of the official A.O.A.C. method (1), which he stated gave a stable color. He claims that both the stability and color intensity with the A.O.A.C. method are greatest when the final pH of the colored system is between 4.0 and 4.7, and proposes use of a sodium succinate buffer in preference to the sodium sulfite recommended in the official method.

Although the work described here was started to check Stoloff's conclusions, the preliminary results led to study of the A.O.A.C. method.

## EXPERIMENTAL WORK

**APPARATUS AND SOLUTIONS.** Transmittancy measurements were made in 1.000-cm. cells with a General Electric recording spectrophotometer adjusted for a spectral band width of 10 m $\mu$ . All pH determinations were made with a glass electrode. Since error due to small amounts of silica in the reagents was always possible, and since the molybdate reagent possessed a slight yellow color, the blanks in the reference beam of the spectrophotometer contained compensating amounts of such materials.

A standard phosphate solution containing 0.1 mg. of phosphorus per ml. was prepared by dissolving 0.4394 gram of twice-recrystallized potassium dihydrogen phosphate in redistilled water and diluting to 1 liter.

Two ammonium molybdate solutions were prepared, differing only in acidity. The solution recommended by the A.O.A.C. (1) was prepared by dissolving 5.0 grams of c.p. ammonium molybdate,  $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$ , in 60 ml. of water, and adding to this solution one containing 15 ml. of concentrated sulfuric acid in 40 ml. of water. Stoloff's solution (8), 1 N in sulfuric acid, was prepared by dissolving 5.0 grams of c.p. ammonium molybdate in approximately 80 ml. of warm (50° C.) water, adding 2.8 ml. of concentrated sulfuric acid to the cooled solution, and diluting to 100 ml. with water. This solution should not be used if a white residue has settled from it.

To stabilize the 0.5% solution of hydroquinone, one drop of concentrated sulfuric acid was added per 100 ml. of solution. Two sodium sulfite solutions were prepared. One contained 200 grams and the other 110 grams of c.p. sodium sulfite per liter of solution. The former has been designated by some as a 20% solution; although this designation is not strictly correct, it was retained and extended to the other reagents.

A sulfite-carbonate solution was made by dissolving 160 grams of c.p. sodium carbonate in 800 ml. of water, adding a solution of 30 grams of c.p. sodium sulfite in 150 ml. of water, and diluting the whole to 1 liter.

A solution of sodium acetate was prepared by dissolving 200 grams of c.p. sodium acetate trihydrate in water and diluting to 1 liter. A solution of sodium succinate (E.K. No. 1219) was prepared similarly. An 0.8 M solution of boric acid was made from recrystallized acid.

To observe the effect of 60 diverse ions on the color reaction, standard solutions were prepared containing 10 mg. of the ion per

ml. Nitrate, sulfate, and acetate salts were used for the cation and sodium, potassium, and ammonium salts were used for the anions.

**STABILITY OF SOLUTIONS PREPARED BY A.O.A.C. METHOD AND STOLOFF'S MODIFICATION.** To check the A.O.A.C. method, a series of molybdenum blue solutions of varying phosphorus concentration was prepared following A.O.A.C. directions (1). Transmittancy curves of the solutions were determined 0.5 hours after the addition of the reductant, and at definite time intervals thereafter. The solutions show some increase in color intensity on standing. Although the error thus produced is not serious it seemed that more stable solutions might be found.

A similar experiment, using Stoloff's modification of the A.O.A.C. method, showed the color to be slightly more stable than that secured by the A.O.A.C. method. The maximum color is not developed, however; when it is developed by addition of more succinate solution, the color fades rapidly.

Stoloff states that 3 ml. of 20% sodium sulfite may be substituted for the sodium succinate in his modified procedure. Maximum color is so developed, but the solutions fade rapidly.

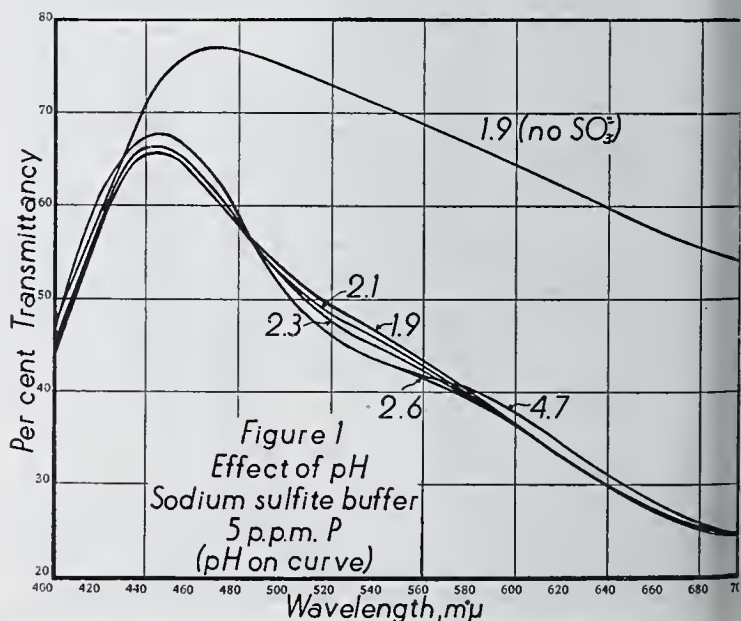
The data in Table I show, in terms of per cent transmittancy at 650 m $\mu$ , the relative stability of three concentrations of solutions prepared under four different sets of conditions.

**EFFECT OF pH ON STABILITY OF COLOR.** The following experimental procedure was used to study the effect of the final pH of the colorimetric stability of the molybdenum blue solutions.

Table I. Stability of Solutions Prepared under Different Conditions

Procedure	Time Hours	pH	Transmittancy		
			2 p.p.m. P %	5 p.p.m. P %	10 p.p.m. P %
A.O.A.C.	0.5	1.6	60.4	27.5	7.2
(sulfite)	4	1.6	59.0	25.9	5.9
Stoloff	0	2.3	73.0	49.2	28.7
(succinate)	4	2.3	72.5	48.0	26.9
	0	4.7	...	...	10.0
	4	4.7	...	...	20.4
Stoloff	0	6.5	62.5	31.4	10.0
(sulfite)	4	6.5	73.0	48.7	30.0
Recommended	0	3.5	62.4	30.5 <sup>a</sup>	9.5
(sulfite)	4	3.5	62.4	30.1 <sup>a</sup>	9.3

<sup>a</sup> pH was 4.5 for this concentration.





Five milliliters of the phosphate solution were pipetted into a 100-ml. volumetric flask, followed by about 25 ml. of water and 8 ml. of the 5% molybdate solution 1 N in acid. This solution was thoroughly mixed and allowed to sit a few minutes. After adding 1 ml. of hydroquinone solution, mixing, and allowing the system to stand a short time, the desired amount of buffer was added, the solution was diluted to the mark with water, and the spectral transmission curve was determined. The pH was then measured, and the remainder of the solution allowed to stand measured intervals of time, after which either the entire transmittancy curve or the transmittancy at 650  $m\mu$  was redetermined.

When sodium sulfite was used as a buffer, 30 minutes were allowed to elapse between addition of the reductant and measurement of the transmittancy curve. In all other cases the transmittancy curve was determined immediately after final dilution.

**Sodium Sulfite.** Solutions prepared with and without small amounts of sulfite are markedly different in color, although their pH is the same (Figure 1). Solutions prepared without sulfite have a greenish-blue color, and their transmittancy curves show a maximum at 465  $m\mu$ . Solutions prepared with sodium sulfite have a much more intense blue color, and the maximum in the transmittancy curve shifts to 445  $m\mu$ . If the amount of sulfite is increased, the color becomes slightly more intense, the pH increases, and the solutions become more stable. Table II shows the stability of these solutions at various pH values. The high pH values were secured with the sulfite-carbonate reagent recommended by Bell and Doisy (2).

This series of experiments reveals a range of color stability from pH 2.3 to 5.2 for solutions standing 1.5 hours, and from pH 2.3 to 5.7 for solutions standing 4.5 hours. The color intensity of all solutions containing sulfite ion is practically the same from pH 2.9 to 6.0. Above pH 6.0 the color intensity increases with increasing pH.

**Sodium Succinate.** In a similar series of experiments with sodium succinate as buffer the range of best color stability was from pH 2.1 to 2.7 for solutions standing one hour. Maximum color development occurs at pH 4.1 to 4.9. Solutions having a pH below 2.1 or above 2.9 fade rapidly on standing. Their color changes gradually from a blue-green to deep blue as the pH changes from 2.1 to 4.1.

**Sodium Acetate.** Solutions prepared with this buffer show transmittancy curves and fading characteristics similar to those obtained using sodium succinate.

**Other Salts.** Similar studies were carried out with such buffers as sodium carbonate and potassium hydrogen phthalate. In

Table II. Effect of Sodium Sulfite on Color Stability

(5 p.p.m. of phosphorus present throughout)

Solution <i>Ml.</i>	pH	Phosphorus Found <sup>a</sup>	
		1.5 hours <i>P.p.m.</i>	4.5 hours <i>P.p.m.</i>
Sulfite reagent, 20 per cent			
0.0	1.9	5.11	5.67
1.0	1.9	5.25	5.73
2.0	2.1	5.17	5.22
3.0	2.3	5.08	5.11
4.0	2.6	5.05	5.08
5.0	4.7	5.01	5.01
6.0	5.2	5.08	5.29
7.0	5.5	5.13	5.62
8.0	5.8	4.65	4.14
9.0	6.1	4.52	3.73
10.0	6.2	4.54	3.51
Sulfite-carbonate reagent			
5.0	6.1	4.66	3.85
7.5	7.1	4.55	3.55
10.0	8.7	4.10	2.18
12.5	9.1	3.85	2.13
15.0	9.3	3.47	1.64
25.0	9.7	3.53	1.81
50.0	9.9	4.08	1.88

<sup>a</sup> Calculations based on readings at 650  $m\mu$ . Transmittancy readings taken 0.5 hour after addition of reductant were assumed to represent entire concentration of phosphorus.

general, they did not permit the gradual changes in pH possible with the other substances, or did not cover the entire pH range. The data secured, however, showed that these substances affected the stability of molybdenum blue in much the same manner as the sodium succinate and sodium acetate.

Of the various buffers tried, sodium sulfite gave the most stable solutions, and was consequently chosen for use in the remainder of the work. Subsequent investigation showed that solutions prepared with sodium sulfite develop their full color within 5 minutes if the pH is between 4.0 and 4.7. Solutions having pH 3.0 to 4.0 increase in color intensity slowly through the first half hour. Although the error due to this increase is small, it becomes negligible if the color measurements are made at a definite time ( $\approx 5$  minutes) after reduction. Below pH 3.0, the solutions do not develop their full color for 0.5 hour, and color measurements should not be made before that time.

**EFFECT OF VARIABLES OTHER THAN pH.** In the study of other variables, the solution containing the phosphorus was pipetted into a 100-ml. volumetric flask, the diverse ion was added next, and then enough water to make the total volume 30 to 40 ml. After this, there were added in order and with mixing, 10 ml. of the 5% ammonium molybdate 1 N in sulfuric acid, 10 ml. of the hydroquinone solution, and 10 ml. of the 11% sodium sulfite solution. The solution was thoroughly mixed after dilution to the mark. The spectral transmission curve and pH were then determined.

Normally this procedure gave solutions with a pH of about 3.5. The exact pH varies slightly because of small errors in the addition of reagents and small differences in the strength of different lots of reagent. It is desirable to check the pH of the solutions occasionally, and if the proper value is not being secured, to make suitable adjustments in the strength or amount of reagent added.

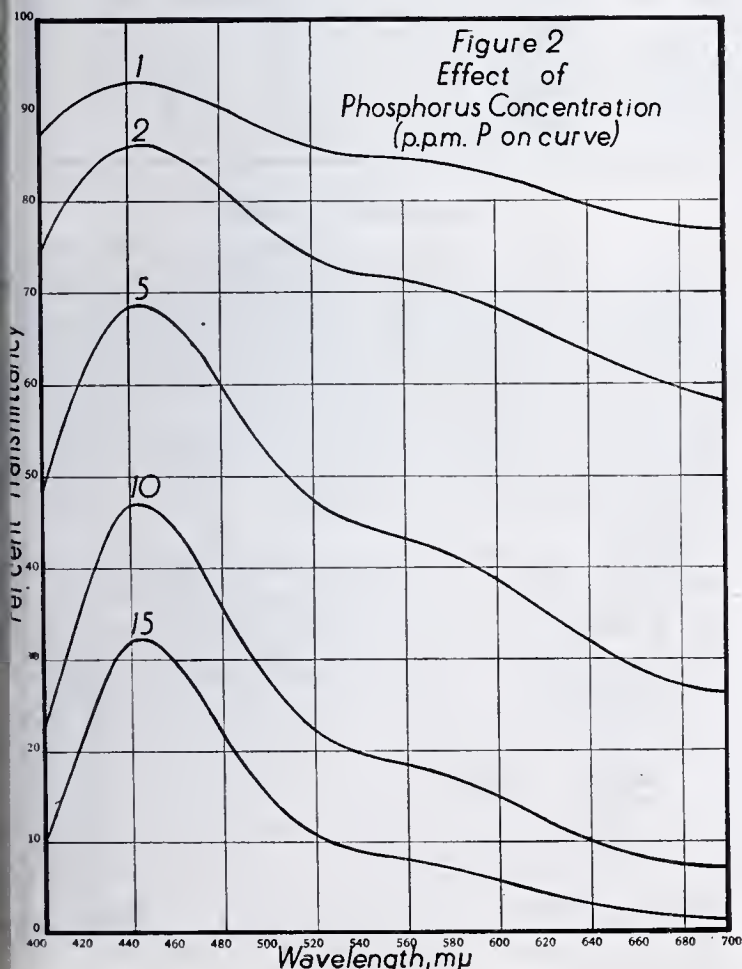
**Molybdate Concentration.** The molybdate concentration was varied, without affecting the pH, by the use of two solutions, one containing 5.0 grams of ammonium molybdate in 100 ml. of solution, the other 1 N in sulfuric acid; 10 ml. of the latter solution were used in all cases. The amount of molybdate was varied from 5 to 15 ml. without any effect on the color produced.

**Hydroquinone Concentration.** Variation in the amount of hydroquinone from 5 to 15 ml. produced no change in the color.

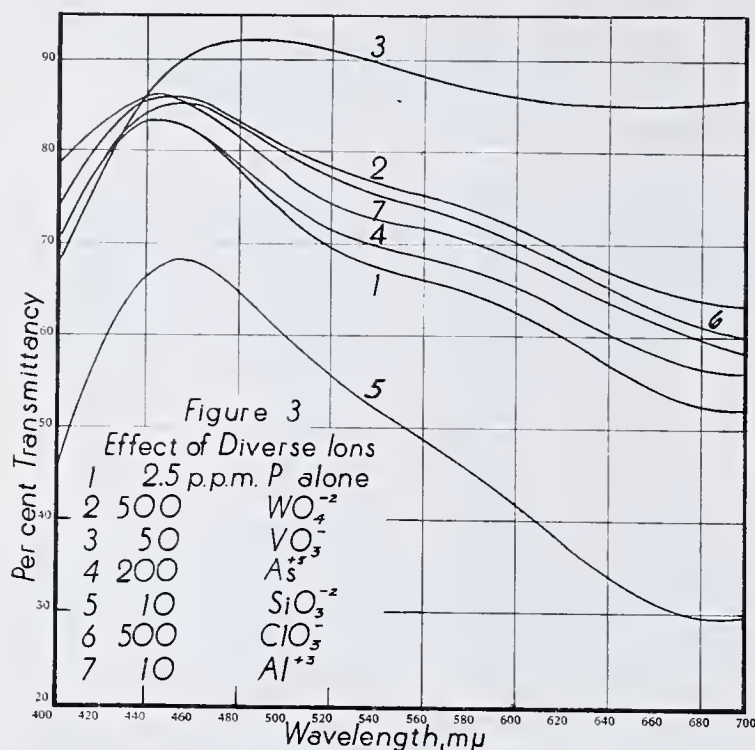
**Sulfite Concentration.** Except for the expected effect on the pH, variations in the amount of sulfite between 8 and 12 ml. have no effect on the color produced.

**Order of Addition of Reagents.** The order of addition of any of the reagents except the sulfite has little effect on the final color. The sulfite must be added last; otherwise the full color is not developed.

**Time between Addition of Reagents.** As much as 5 minutes may elapse between the addition of each reagent without affecting







the color, provided blanks are used. If blanks are not used, the sulfite should be added immediately after the hydroquinone. In the absence of sulfite, the hydroquinone slowly reduces the molybdate and introduces a positive error.

**Stability.** The stability of these solutions over short periods of time has been discussed. Solutions allowed to stand in glass-stoppered bottles for 3 months show considerable increase in color intensity and a change in color. The maximum in the transmittancy curve shifts toward the purple on standing.

**Heating the Solution.** If the solutions are heated in boiling water after color development, the color intensity increases. The amount of increase is dependent on the length of time of heating. The resulting solutions are no more stable than unheated solutions. Although greater sensitivity could be secured by heating the solutions, the increase was not thought large enough to justify the complications introduced into the procedure.

**Phosphorus Concentration.** The range of phosphorus concentration which can be measured in 1-cm. thickness by this procedure is shown in Figure 2. Beer's law is valid over the entire range for transmittancy measurements at 650  $m\mu$ .

**Diverse Ions.** In observing the effect of diverse ions, 2.5 p.p.m. of phosphorus were used. The transmittancy curve of the solution was drawn within 5 minutes after color development, and the apparent phosphorus concentration calculated from transmittancy measurements made at 650  $m\mu$ . In keeping with previous studies of this type, a 2% error was assumed to be negligible.

An error not exceeding 2% is caused by 500 p.p.m. of each of the following ions: beryllium, cadmium, calcium, lithium, magnesium, manganese, potassium, sodium, uranyl, acetate, benzoate, borate, bromide, carbonate, chloride, cyanide, formate, iodide, lactate, oxalate, perchlorate, salicylate, sulfate, tartrate, thiocyanate, and thiosulfate.

The following metals precipitate under the conditions used: barium, bismuth, gold, lead, mercury (-ic or -ous), silver, stannic, strontium, thorium, titanium, and zirconium.

Strong oxidizing agents, such as dichromate and permanganate, oxidize the reducing agents faster than the molybdiphosphoric acid; and strong reducing agents, such as sulfide and chlorostannous ions, reduce the excess molybdate reagent to molybdenum blue. Therefore, all strong oxidizing and reducing agents must be absent.

Silicates, arsenates, and arsenites form heteropoly acids with acid molybdate solutions just as phosphorus does. Molybdosilicic acid is readily reduced under the conditions used here, and silicates must be entirely absent. The heteropoly acid containing arsenic is not so readily reduced, and 100 p.p.m. of arsenate or 50 p.p.m. of arsenite may be present without interference.

The effect of other diverse ions is summarized in Table III, the calculations being made as described earlier (?). A few curves are shown in Figure 3.

**Removal of Fluoride Interference.** Although fluoride interference is not so serious in this procedure as in other molybdenum blue methods (9), it can be readily avoided with boric acid, as

described by Kurtz (6). Five milliliters of 0.8 *M* boric acid added before the color development, prevent the interference of 1000 p.p.m. of fluoride. The color can be developed immediately after the addition of boric acid. The use of larger amounts of boric acid to remove larger amounts of fluoride ion leads to small negative errors, the magnitude of the error increasing slightly with increasing boric acid and/or fluoride concentration.

## DISCUSSION

Table II shows the peculiar effect of raising the pH of these solutions above 5.8. Below this value the color intensity of the solutions either is constant or increases gradually on standing. Above pH 5.8, the solutions fade on standing. Similar results were obtained with 1-amino-2-naphthol-4-sulfonic acid as reductant, following the procedure of Fiske and Subbarow (3). If the solutions listed in Table II are heated in boiling water for 30 minutes, those with pH 5.6 or lower retain their blue color while those with pH 5.9 or higher give a colorless solution at low phosphorus concentration, or an orange-red precipitate at higher phosphorus concentrations. A solution with pH 5.8 showed slight blue color and a reddish turbidity.

The exact cause of this behavior is not known; it is probably connected with the changes in conductance of molybdiphosphoric acid solutions noted by Jander and Witzmann (5) at pH 5.5 to 6.5.

Table III. Effect of Diverse Ions

Ion	Added as:	Present P.p.m.	Error %	Amount Permissible P.p.m.
$\text{Al}^{+++}$	$\text{Al}(\text{NO}_3)_3$	10	19	0
$\text{Ce}^{++++}$	$(\text{NH}_4)_2\text{Ce}(\text{NO}_3)_6$	100	32	0
$\text{Co}^{++}$	$\text{Co}(\text{NO}_3)_2$	100	2	100
$\text{Cr}^{+++}$	$\text{Cr}_2(\text{SO}_4)_3$	25	0	25
$\text{Cu}^{++}$	$\text{CuSO}_4$	25	0	25
$\text{Fe}^{+++}$	$\text{Fe}_2(\text{SO}_4)_3 \cdot (\text{NH}_4)_2\text{SO}_4$	10	12	0
$\text{Fe}^{++}$	$\text{FeSO}_4 \cdot (\text{NH}_4)_2\text{SO}_4$	100	3	50
$\text{Ni}^{++}$	$\text{Ni}(\text{NO}_3)_2$	100	0	100
$\text{AsO}_3^{---}$	$\text{Na}_2\text{HAsO}_3$	50	0	50
$\text{AsO}_4^{---}$	$\text{H}_3\text{AsO}_4$	100	0	100
$\text{B}_4\text{O}_7^{--}$	$\text{Na}_2\text{B}_4\text{O}_7$	50	2	50
$\text{C}_6\text{H}_5\text{O}_7^{---}$	$\text{Na}_3\text{C}_6\text{H}_5\text{O}_7$	300	2	300
$\text{ClO}_3^-$	$\text{KClO}_3$	10	0	10
$\text{F}^-$	$\text{NaF}$	200	3	100
$\text{IO}_2^-$	$\text{KIO}_3$	10	0	10
$\text{IO}_4^-$	$\text{KIO}_4$	300	2	300
$\text{NO}_2^-$	$\text{KNO}_2$	100	1	100
$\text{P}_2\text{O}_7^{---}$	$\text{Na}_4\text{P}_2\text{O}_7$	10	3	5
$\text{PtCl}_6^{--}$	$\text{H}_2\text{PtCl}_6$	500	New hue	0
$\text{SeO}_4^{--}$	$\text{Na}_2\text{SeO}_4$	100	0	100
$\text{VO}_3^-$	$\text{KVO}_3$	10	60	0
$\text{WO}_4^{--}$	$\text{Na}_2\text{WO}_4$	100	10	20

## RECOMMENDED PROCEDURE

**SAMPLE.** From a representative sample, suitably prepared measure a sample containing at least 0.002 mg. of phosphorus and dissolve it (if necessary) by appropriate means. Obviously dissolution in phosphoric acid, or fusion with pyrophosphate, is inapplicable. Care should be taken to have the phosphorus in the form of orthophosphate. Interfering ions must be removed or their action inhibited to bring their effective concentrations within the limits given in Table III. Make the resulting solution just acidic to litmus, and dilute to a definite volume in a volumetric flask. (In case of very small amounts of phosphorus it may be necessary to use all the sample. This step would then be omitted.)

**DESIRED CONSTITUENT.** Transfer a suitable aliquot of this solution to a 100-ml. volumetric flask. If Nessler tubes are used for visual comparison, the aliquot should contain 0.002 to 0.20 mg. of phosphorus. For photometric measurement with 1-cm. cells, the aliquot should contain 0.1 to 1.5 mg. of phosphorus. Add enough water to make the total volume at least 30 ml. (Molybdiphosphoric acid precipitates unless water is added. In case of precipitation, increase the amount of water. If fluoride is present, add 5 ml. of 0.8 *M* boric acid at this point. If more than 1000 p.p.m. of fluoride ion is present, remove it by fuming with sulfuric acid. Too much boric acid causes a small negative error.) Add in order, and with constant mixing, 10 ml. of 5% ammonium molybdate solution 1 *N* in sulfuric acid, 10 ml. of 0.5% hydroquinone solution, and 10 ml. of 11% sodium sulfite solution. Dilute to the mark, and mix well. The final pH of the solution should be between 3.0 and 4.7; otherwise make suitable adjustment in the amount of the molybdate or sulfite



reagent. Allow the solution to stand 30 minutes, and then measure the color by any of the usual means. (Measurement can be made within the first half hour after color development, provided the time of measurement is controlled within  $\pm 5$  minutes.)

Visual comparison may be made with standards prepared with known amounts of phosphorus, or with a series of permanent standards (9). Filter photometric measurement should be made with a red filter. For spectrophotometric measurement in the visual region a wave length between 600 and 700  $m\mu$  seems preferable. Measurement may be made beyond the color range if a suitable spectroradiometer is available. Thus, Fontaine (4) shows a peak in the absorption band near 820  $m\mu$  for solutions reduced with chlorostannous acid. The writers' solutions did not show this band, but one much sharper was found with a minimum near 340  $m\mu$ .

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ABSTRACTED from a thesis presented by R. E. Kitson to the Graduate School of Purdue University in partial fulfillment of the requirements for the degree of doctor of philosophy, February, 1944.

Quantitative Determination of Mixtures of

Methyl Vinyl Ketone and Diacetyl

A Dropping Mercury Electrode Method

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A dropping mercury electrode method has been described for determination of (1) methyl vinyl ketone alone and in mixtures with methyl vinyl carbinol and methyl ethyl ketone and (2) diacetyl alone and in mixtures with methyl vinyl ketone and methyl ethyl ketone.

STUDIES are in progress in these laboratories on the catalytic vapor-phase oxidation of methyl vinyl carbinol and of 2,3-butylene glycol. In the course of this work it became necessary to devise methods for determining methyl vinyl ketone in the presence of methyl vinyl carbinol and methyl ethyl ketone, and diacetyl in the presence of methyl vinyl ketone and methyl ethyl ketone. The present communication presents such methods using the dropping mercury electrode. The methods are based on the fact that methyl vinyl ketone and diacetyl are reduced at the dropping mercury electrode while methyl vinyl carbinol and methyl ethyl ketone are not so reduced.

in an atmosphere of nitrogen. Potassium chloride, 0.1 *N*, was used as the indifferent electrolyte.

SOURCE OF MATERIALS

The methyl vinyl ketone was made by the catalytic vapor-phase oxidation of methyl vinyl carbinol; the details of the method will be made available at a later date. The highly purified methyl vinyl ketone had the following characteristics: b.p. = 79–80° C. at 760 mm.,  $n_D^{20} = 1.4081$ , and  $d_4^{27} = 0.862$ . The diacetyl, purchased from the Eastman Kodak Company, was further purified and had the following characteristics: b.p. = 86.5–87.5° C. at 750 mm.,  $n_D^{20} = 1.3910$ , and  $d_4^{25} = 0.980$ . These constants, for both chemicals, agreed with the best data available in the literature.

The methyl vinyl carbinol was purchased from the Shell Chemical Company and the methyl ethyl ketone from the Eastman Kodak Company. In each case the stock solutions consisted of 1 ml. of the chemical made up to 100 ml. with 0.10 *N* potassium chloride. These solutions were diluted with 0.10 *N*

APPARATUS AND PROCEDURE

The equipment employed was manually operated and was similar to that described by Kolthoff and Lingane (2) and Lingane and Kolthoff (3). The voltage was measured against an external saturated calomel electrode. The current, in microamperes, was calculated from the *IR* drop across a resistance of 7000 ohms. The pressure on the mercury was maintained at 80 cm. of mercury; the drop-time was 3.4 seconds. The value of *m*, in weight of mercury flowing from the capillary per second, was 0.00134. Dissolved air was removed from the solutions by means of gaseous nitrogen and all determinations were made at 25° C.

Table II. Diffusion Currents and Half-Wave Potentials for Methyl Vinyl Ketone

Molarity $\times 10^3$		$E_{1/2}$	Diffusion Current	
Experimental	Calculated Equation 2		Experimental	Calculated Equation 1
0.494	0.493	1.43	1.80	1.79
1.234	1.213	1.42	4.43	4.50
2.468	2.469	1.41	9.01	9.01

Table III. Current-Voltage Data<sup>a</sup> for Mixtures of Methyl Vinyl Ketone, Methyl Ethyl Ketone, and Methyl Vinyl Carbinol

(Molarity $\times 10^3$ )					
MVK = 0.494		MVK = 1.23		MVK = 1.87	
MEK = 0.448		MEK = 1.12		MEK = 0.224	
MVC = 0.469		MVC = 1.17		MVC = 0.234	
Current		Current		Current	
Microamperes	Volts	Microamperes	Volts	Microamperes	Volts
0.50	1.265	0.40	1.200	0.53	1.220
0.53	1.314	0.46	1.283	0.57	1.270
1.57	1.422	0.89	1.352	1.06	1.346
2.33	1.506	2.79	1.433	2.86	1.418
2.33	1.580	4.36	1.490	5.14	1.470
2.33	1.653	5.04	1.557	6.71	1.530
2.33	1.714	4.93	1.621	7.07	1.594
...	...	4.94	1.695	7.71	1.655
...	...	4.93	1.760	7.43	1.725
...	...	...	...	7.37	1.789
...	...	...	...	7.37	1.850

Table I. Current-Voltage Data<sup>a</sup> for Methyl Vinyl Ketone Solutions

(Molarity $\times 10^3$ )					
0.494		1.23		2.47	
Current		Current		Current	
Microamperes	Volts	Microamperes	Volts	Microamperes	Volts
0.56	1.245	0.53	1.250	0.57	1.080
0.57	1.338	0.71	1.330	0.57	1.215
1.31	1.412	1.71	1.400	0.90	1.290
2.21	1.478	3.43	1.454	4.93	1.407
2.46	1.545	4.71	1.515	7.74	1.462
2.46	1.615	5.20	1.581	9.07	1.527
2.36	1.680	4.96	1.650	9.50	1.577
2.36	1.745	4.99	1.718	9.57	1.640
2.36	1.820	4.99	1.800	9.57	1.720

<sup>a</sup> Potential difference between dropping electrode and saturated calomel electrode.

<sup>a</sup> Potential difference between dropping electrode and saturated calomel electrode.



**Table IV. Diffusion Currents for Mixtures of Methyl Vinyl Ketone, Methyl Vinyl Carbinol, and Methyl Ethyl Ketone**Concentration of MVK =  $M \times 10^3$ . Concentrations of MEK and MVC as in Table III)

Experimental	Molarity $\times 10^3$ Calculated Equation 2	Experimental	Diffusion Current Calculated Equation 1
0.494	0.499	1.82	1.80
1.23	1.22	4.47	4.49
1.87	1.91	6.80	6.83

**Table V. Current-Voltage Data<sup>a</sup> for Diacetyl**(Molarity  $\times 10^3$ )

1.136		0.909		0.568		0.454		0.379	
Current Micro-amperes	Volts	Current Micro-amperes	Volts	Current Micro-amperes	Volts	Current Micro-amperes	Volts	Current Micro-amperes	Volts
0.53	0.459	0.46	0.521	0.51	0.484	0.39	0.507	0.39	0.575
0.56	0.517	0.47	0.633	0.51	0.593	0.40	0.615	0.40	0.680
0.57	0.626	0.49	0.675	0.54	0.673	0.43	0.688	0.59	0.786
0.60	0.690	0.59	0.735	0.71	0.774	0.67	0.790	1.17	0.840
1.39	0.788	1.69	0.822	1.97	0.860	1.34	0.834	1.61	0.928
2.29	0.824	3.00	0.905	2.36	0.946	1.94	0.924	1.67	1.020
3.57	0.875	3.23	0.991	2.40	1.036	1.97	1.013	1.69	1.120
3.97	0.963	3.24	1.080	2.41	1.120	...	...	...	...
3.97	1.053	...	...	...	...	...	...	...	...

<sup>a</sup> Potential difference between dropping electrode and saturated calomel electrode.

potassium chloride to give the concentrations used in the experiments.

**ANALYSIS OF MIXTURES**

METHYL VINYL KETONE, METHYL VINYL CARBINOL, AND METHYL ETHYL KETONE. Current-voltage data for three concentrations of methyl vinyl ketone are given in Table I. The diffusion currents,  $dc$ , and half-wave potentials,  $E_{1/2}$ , are given in Table II. In this, and in subsequent tables, the diffusion current is obtained by subtracting the residual current from the limiting current. The diffusion current is a linear function of the molar concentration, the equation being:

$$dc = 3.65 M \times 10^3 \quad (1)$$

from which,

$$M \times 10^3 = 0.274 \times dc \quad (2)$$

Calculations, using the above equations, given in Table II, show that the method is entirely satisfactory for the analysis of methyl vinyl ketone alone.

Current-voltage data for mixtures of methyl vinyl ketone, methyl vinyl carbinol, and methyl ethyl ketone are presented in Table III. The calculated values show that the methyl vinyl carbinol and methyl ethyl ketone are not reduced and do not interfere with the determination of the methyl vinyl ketone.

DIACETYL, METHYL VINYL KETONE, AND METHYL ETHYL KETONE. Adkins and Cox (1) presented data on the reduction of several aldehydes and ketones at the dropping mercury electrode. Using 0.10 *N* ammonium chloride as the indifferent electrolyte, in unbuffered solution, they found two half-wave potentials for diacetyl at 0.93 and 1.68 volts. These values, recalculated on the basis of the use of an external saturated calomel electrode, are 0.84 and 1.59 volts. The authors emphasized the necessity for determining the effect of other solutes on the wave height of the reducible compounds.

Current-voltage data for five concentrations of diacetyl are given in Table V. The diffusion currents,  $dc$ , and half-wave potentials are shown in Table VI. The half-wave potential is 0.83 volt which is identical, within experimental error, with the 0.84 volt calculated from the results of Adkins and Cox (1). The diffusion current is not a linear function of the concentration of the diacetyl but is given by the hyperbolic relation:

$$\log (dc) = 0.8766 \log (M \times 10^4) - 0.3967 \quad (3)$$

from which,

$$\log (M \times 10^4) = 1.1411 \log (dc) + 0.4526 \quad (4)$$

Calculations, using the above equations, presented in Table VI, show the method to be satisfactory for the analysis of diacetyl alone. The fact that the hyperbolic function is also characteristic of adsorption reactions indicates that adsorption phenomena may be operating in the case of diacetyl.

Current-voltage data for four mixtures of diacetyl (DA), methyl vinyl ketone (MVK), and methyl ethyl ketone (MEK) are given in Table VII. Diffusion currents for the mixtures, together with data calculated by Equations 3 and 4, are given in Table VIII. It is evident that the procedure is applicable to mixtures of diacetyl and methyl vinyl ketone in the presence of methyl ethyl ketone, as well as to the diacetyl or methyl vinyl ketone alone.

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**Table VI. Diffusion Currents and Half-Wave Potentials for Diacetyl**

Molarity $\times 10^3$		$E_{1/2}$	Diffusion Current	
Experimental	Calculated Equation 4		Experimental	Calculated Equation 3
1.136	1.133	0.824	3.37	3.38
0.909	0.902	0.830	2.76	2.78
0.568	0.575	0.821	1.86	1.84
0.454	0.454	0.825	1.51	1.51
0.379	0.379	0.826	1.29	1.29

**Table VII. Current-Voltage Data<sup>a</sup> for Mixtures of Diacetyl, Methyl Vinyl Ketone, and Methyl Ethyl Ketone**(Molarity  $\times 10^3$ )

DA = 0.385		DA = 0.462		DA = 0.462		DA = 0.923	
MVK = 0.834		MVK = 0.501		MVK = 0.750		MVK = 0.250	
MEK = 0.834		MEK = 0.501		MEK = 0.750		MEK = 0.250	
Current Micro-amperes	Volts	Current Micro-amperes	Volts	Current Micro-amperes	Volts	Current Micro-amperes	Volts
0.37	0.480	0.31	0.526	0.47	0.459	0.39	0.475
0.39	0.590	0.36	0.633	0.53	0.576	0.41	0.580
0.41	0.695	0.43	0.700	0.60	0.680	0.44	0.685
0.67	0.791	1.36	0.807	0.89	0.792	0.50	0.733
1.21	0.843	1.93	0.873	1.93	0.885	0.64	0.772
1.71	0.931	2.00	0.965	2.10	0.975	2.37	0.845
1.79	1.023	1.96	1.060	2.14	1.067	3.07	0.936
1.77	1.110	1.96	1.140	2.14	1.152	3.14	1.020
1.77	1.200	1.94	1.225	2.14	1.240	3.14	1.108
1.77	1.281	2.00	1.290	2.16	1.322	3.13	1.193
1.77	1.335	2.71	1.369	2.43	1.400	3.13	1.277
2.36	1.411	3.57	1.439	3.61	1.466	2.13	1.355
3.83	1.470	3.77	1.506	4.60	1.528	3.31	1.433
4.56	1.533	3.77	1.577	4.81	1.600	3.86	1.500
4.73	1.590	3.77	1.649	4.81	1.705	3.97	1.573
4.74	1.668	...	...	...	...	3.97	1.642
4.74	1.735	...	...	...	...	...	...

<sup>a</sup> Potential difference between dropping electrode and saturated calomel electrode.**Table VIII. Diffusion Currents of Mixtures of Diacetyl, Methyl Vinyl Ketone, and Methyl Ethyl Ketone**

Diacetyl				Methyl Vinyl Ketone			
Molarity $\times 10^3$		Diffusion Current		Molarity $\times 10^3$		Diffusion Current	
Experimental	Calcd. Eq. 4	Experimental	Calcd. Eq. 3	Experimental	Calcd. Eq. 2	Experimental	Calcd. Eq. 1
0.385	0.402	1.36	1.31	0.834	0.814	2.97	3.04
0.462	0.462	1.53	1.53	0.501	0.501	1.83	1.83
0.462	0.463	1.54	1.53	0.750	0.732	2.67	2.74
0.923	0.879	2.70	2.81	0.250	0.230	0.84	0.91



# Delivery of Liquids at Low and Constant Rates

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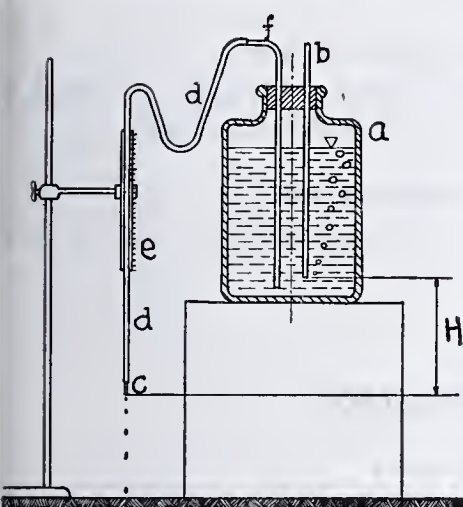


Figure 1

CONSIDERABLE interest has been evinced recently in the problem of delivering liquids, on a laboratory or pilot-plant scale, at constant rates of flow (1).

A device successfully used by the writer over a period of years, and for varying utilizations, may be of interest because of its relative simplicity, ease of construction, continuity of operation, and ready adjustment to varying rates of flow.

The well-known Mariotte bottle is the basis of the delivery device, and only standard laboratory equipment is needed for its construction and operation. Highly precise regulations of flow rates are feasible, as little as 0.06 ml. per minute having proved practical. Pure liquids, solutions of solids, colloidal solutions, and even relatively coarse suspensions lend themselves to the procedure, a dilute drilling mud representing, in actual practice, one of the materials so handled. If continuous operation is desired, or if large quantities of liquid must be run, simple modifications allow maintaining steady flow without breaking the continuity of flow during refilling.

Figure 1 represents the simplest form of the device, for use where there is no need for refilling during operation. The Mariotte bottle, *a*, acts as a constant-head reservoir. It consists of any suitable bottle, tightly stoppered, with two glass tubes passing through the closure. Air enters through tube *b*, which ends near the bottom of the bottle, the end being turned at a right angle, if desired, particularly for low rates of flow. The other glass tube, *f*, is directly connected through a length of soft-rubber tubing, *d*, to orifice *c*, the rubber tubing being encased, part of the way, by glass tubing *e*, fitting around it snugly. This combination enables exact vertical adjustment which can be accomplished easily by pulling the rubber tubing up or down, through the supporting glass tube, *e*, until *c* is at any desired height.

Liquid flowing through the tube and orifice operates under a liquid head, *H*, which represents the distance between the end of *b* and the end of *c*. Since the rate of flow is a function of *H*, rate changes can be accomplished easily and accurately by adjusting the level of *c* by sliding rubber tube *d* up or down through *e*, or by adjusting the level of the whole assembly on a ring stand.

To obtain various ranges of flow rate, orifices of varying lengths and diameters may be used. After appropriate calibration, a scale may be positioned behind *e* to facilitate operations and reproducibility.

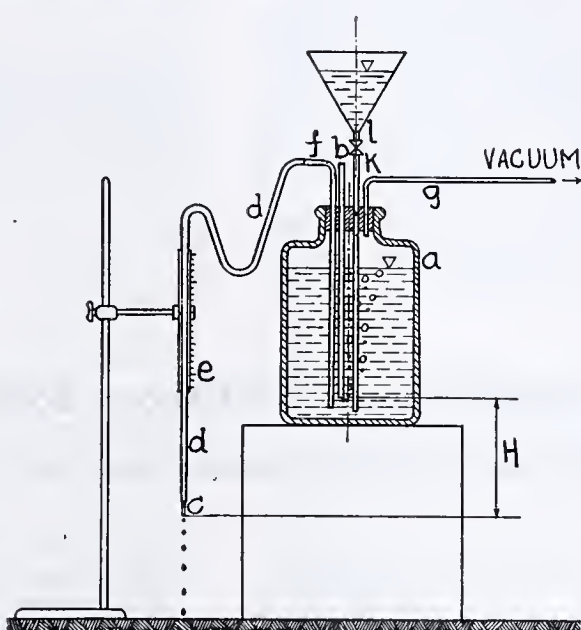


Figure 2

It has been found, in practice, best to make the device continuous, or to add liquid without interrupting outflow. One such modification is shown in Figure 2, another in Figure 3.

In Figure 2, a third glass tube, *g*, passes through the closure into the space above the liquid, being directly connected to a source of vacuum (the vacuum should be sufficient to draw in air through *b*, but not high enough to cause the liquid to boil). Replenishment of the liquid is accomplished, when desired, through a fourth tube, *k*, by opening stopcock *l*; the tube may be directly connected to a funnel containing the additional liquid or may be connected more elaborately to a larger holding vessel, flowing by gravity or by siphon.

When it is inconvenient to have the liquid holding vessel at a level above the constant-head reservoir, an arrangement such as shown in Figure 3 may be used; this is particularly valuable when large quantities of liquid are involved. Compressed air acts to lift the liquid to above the reservoir level, from which point it flows into the reservoir through tube *k*. Vent *m* represents a stopcock or valve which must be opened whenever the supply in the holding vessel is replenished through funnel *n*; otherwise it is kept closed, as is also the stopcock in funnel *n*.

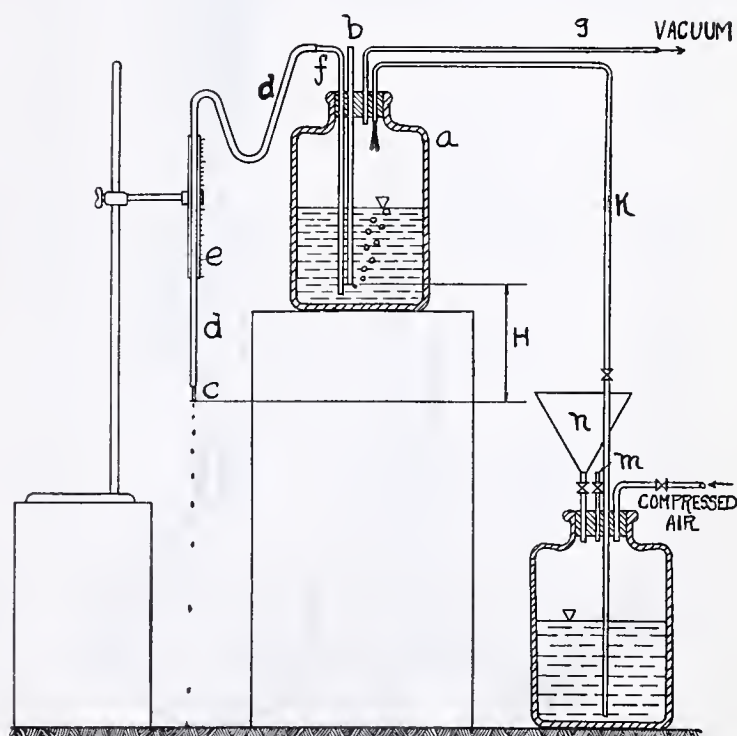


Figure 3

<sup>1</sup> Deceased.



It may be of theoretical interest to indicate the reasons why a uniform rate is maintained despite vacuum and air pressure conditions in various parts of the system. The air space above the liquid in the Mariotte bottle reservoir is under vacuum equivalent to the hydrostatic head between the surface of the liquid on the one hand, and the end of the air tube, *b*, on the other hand. If liquid were to be added directly through a tube and funnel, without the use of a vacuum pump, the air would be compressed and no air would be drawn in through *b*; accordingly, equilibrium conditions would be disturbed. However, if during refilling a vacuum pump is attached to the air space of the reservoir, as indicated in Figures 2 and 3, the volume of air drawn out of the bottle will be greater than the volume of liquid drawn in, any difference being amply taken care of by the air intake through *b*.

If the liquid used is suspected of containing suspended material that may clog the orifice, a filter (such as a glass wool plug) may

be inserted between the end of the glass tube, *f*, and the rubber hose, *d*.

When heavy suspensions, on the other hand, are to be handled, all tubes and tubing should be as wide as possible, with hydrostatic head, *H*, kept correspondingly low.

These devices have all been in active operation in conjunction with small-scale continuous water-softening tests and in studying particle size distribution on diluted oil-well-drilling muds.

#### ACKNOWLEDGMENT

The help of George H. Fancher of the University of Texas is gratefully acknowledged in many phases of this work.

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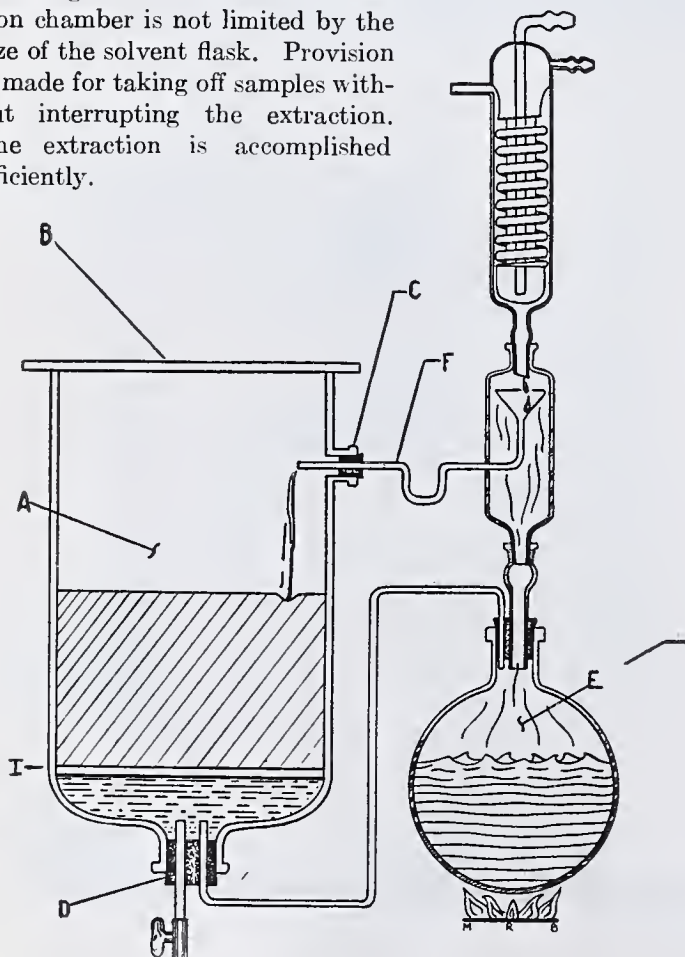
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## A Large-Capacity Continuous Solids Extractor

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OF THE innumerable designs which have been published for glass extraction apparatus (5), very few are adaptable to large-scale work because of their expensive and unwieldy construction. Large-capacity extractors of simple design have been described by Beal (1), Drake and Spies (2), Smallwood (6), and Jonnard (4). While these can all be made from standard laboratory glassware, they suffer minor disadvantages which limit their usefulness.

The apparatus described below has been designed to overcome these disadvantages. It can be constructed from standard, readily available glassware in sizes up to 22 liters. It can be charged and emptied without dismantling. The size of the extraction chamber is not limited by the size of the solvent flask. Provision is made for taking off samples without interrupting the extraction. The extraction is accomplished efficiently.



The basic constituents of the extractor are a glass bottle, a condenser, and a flask.

The extraction chamber is made from an inverted Pyrex bottle, *A*, by cutting off the bottom and grinding the rim to fit smoothly to a ground-glass plate which acts as a cover, *B*. A hole is drilled in the side of the bottle 2 to 3 cm. below the cover and a tubulature is welded on at *C*. (An aspirator bottle with tubulature may be used if available in the desired size.)

The solvent is vaporized in the flask, *E*, and condensed by the condenser, from which it flows through *F* into the extraction chamber. The extract returns to the flask by means of a glass tube which is inserted through a cork, *D*, at the bottom of the extraction chamber and bent so that when the extraction chamber is filled, the condensation of additional solvent will cause an overflow back to the flask. The connection at *F* may be made by semiball joints or by Thiokol tubing. If desired, the corks at *C* and *D* may be replaced by all-glass joints.

Samples may be taken off from time to time by means of a stopcock at *D*.

**CHARGING.** The extractor is charged by removing the ground-glass plate cover, *B*, and placing a bag containing the material to be extracted on the perforated porcelain plate, *I*. The extraction chamber is filled with solvent and then a sufficient excess is added so that the overflow fills the flask to a desired height. The cover is replaced and the extractor is ready for operation.

**EXTRACTION.** The flask is heated and the condensed solvent enters the extraction chamber, displacing the extract through the return tube back into the flask.

The material is extracted by constant percolation and soaking instead of repeated exhaustion as in the Soxhlet apparatus. This method of continuous removal of the solvent from the bottom of the extractor at the same rate that fresh solvent enters the top has been shown to be decidedly more efficient than the Soxhlet exhaustion method (3). Furthermore, the volume of the flask is limited only by the rate at which one wishes to distill and the flask does not have to be large enough to receive the entire contents of the extraction chamber as in the Soxhlet type of apparatus. Thus a relatively small amount of solvent may be heated at one time.

To empty the extraction chamber, the residual solvent is withdrawn through the stopcock at *D* and the spent charge is removed through the top.

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# Laboratory Continuous Countercurrent Liquid-Liquid Extractor

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THE inefficiency of the ordinary types of laboratory extraction apparatus makes the recovery of solutes from solutions by extraction with immiscible solvents a very time-consuming operation. In the conventional type of laboratory extractor the immiscible solvent is vaporized in the boiling flask, condensed in a single upright condenser placed above the flask containing the solution to be extracted, and allowed to run down a funnel tube fitted at the bottom with a diffuser plate. The diffuser plate produces a fine dispersion of the immiscible solvent which rises through the solution to be extracted and flows back to the boiling flask through a suitable overflow arm. The inefficiency of this type of extractor is due to a low rate of solvent flow or lack of intimate contact between solvent and solution. The use of a diffuser plate of low porosity in order to obtain intimate liquid-solvent contact necessarily limits the rate of solvent flow; the interference between ascending vapor and descending liquid in the condenser limits the rate at which the solvent may be vaporized and condensed.

To increase the efficiency of liquid-liquid extraction an improved type of extractor was designed by Hossfeld (1) which is equipped with separate arms for vapor outlet and solvent return, thus affording a high rate of solvent flow. The centrifugal dispersion of the solvent and the agitation of the solution in the Hossfeld apparatus result in intimate contact between solvent and solution. The Hossfeld apparatus is a definite advance over the conventional laboratory extractor.

A new type of laboratory countercurrent extractor has been designed and used extensively in these laboratories for the recovery of 2,3-butyleneglycol and acetylmethylcarbinol from fermentation mashes and mash concentrates by extraction with diethyl ether. The unit employed has been found more efficient than any other type of extractors tested, and is diagrammed in Figure 1.

The column is 1220 mm. in length and 45 mm. in diameter. The fermentation mash solution to be extracted is placed in the 4-liter Erlenmeyer flask shown at the left of the diagram and a layer of this solution is also placed in the bottom of the extraction column as shown. The column is then filled with solvent and about 1.5 liters of solvent are placed in the 3-liter boiling flask shown at the right. The vaporized solvent is condensed by the Allihn condensers, flows down through the tube at the right, enters the bottom of the extraction column, flows upward through the column, and returns to the boiling flask through the overflow arm. The mash is forced by the circulating pump out of the mash flask into the top of the extraction column through the jet, which is merely a piece of glass tubing constricted at the lower end. The interfacial tension and the pressure on the liquid produce dispersion into droplets at the tip. When a jet of the proper size is used, the pressure forces a fine spray of the liquid into the column of solvent. The droplets of solution then fall through the solvent to the bottom of the column. The solution is pumped to the mash flask and continuously recirculated. The rate of ether flow in this unit is 2.0 liters per hour and the rate of mash circulation is 4.2 liters per hour. Rubber connections in the unit are coated with water glass to prevent solvent loss, and all ground-glass joints are standard taper 24/40.

The purpose of the two condensers is to ensure a more complete condensation of the solvent. This is a particularly important consideration when diethyl ether is used as the solvent. The use of an angle condenser before the upright condenser allows a higher rate of solvent flow, since there is then less turbulence at the lower tip of the upright condenser. In ether extractions where emulsions are not encountered, placing a finger cut from a rubber glove or a rubber finger-stall over the top of the upright condenser prevents much of the ether loss which would otherwise occur. The extractor has been operated continuously for 3 days

under these conditions at an ether flow rate of 2.0 liters per hour with no noticeable loss of ether.

In extractions of fermentation liquors, emulsion formation is sometimes encountered. Although the emulsion-forming constituents can frequently be removed by treatment with basic lead acetate or similar precipitating agents, this treatment also often removes considerable quantities of the constituent to be recovered. Whenever a tendency toward emulsion formation was noted in the extraction column it was effectively overcome by passing a slow stream of air upward through the column.

The circulating pump employed for circulation of the solution is diagrammed in detail in Figure 2. The spool, *B*, bearing the rollers, *A*, rotates within the ring, *C*, which is welded to the plate, *D*. The rollers press against the rubber tubing, *E*, and force the liquid through. The tubing is held against the rollers by the sliding block, *F*, and the pressure between rollers and tubing is adjusted by screws, *H*, on the U-clamps, *G*. Grooves are cut in the ends of ring *C* and block *F* to hold the tubing in place. The tubing used is 9 mm. in diameter. The spool diameter is 7.5 cm. (3 inches) and the rollers are 0.93 × 1.56 cm. ( $\frac{3}{8} \times \frac{5}{8}$  inches). The roller spool is driven at 50 r.p.m.

The extraction rate of the extractor described above was compared with the rates for two other types of extractors. No. 1 was the conventional type of laboratory extractor, procured from a laboratory supply company, consisting of a 4-liter bottle fitted with a stirrer, a sintered-glass gravity-fed dispersing plate, and the usual upright Allihn condenser and boiling flask. Extractor 2 was constructed according to the specification of Hossfeld (1).

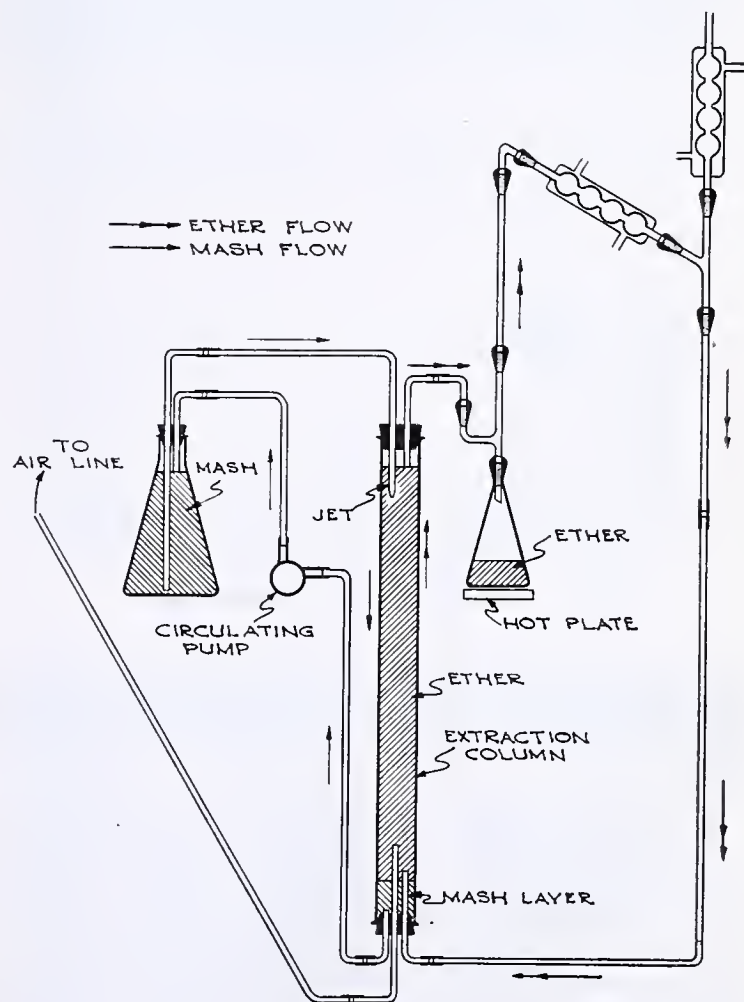


Figure 1. Countercurrent Extraction Apparatus



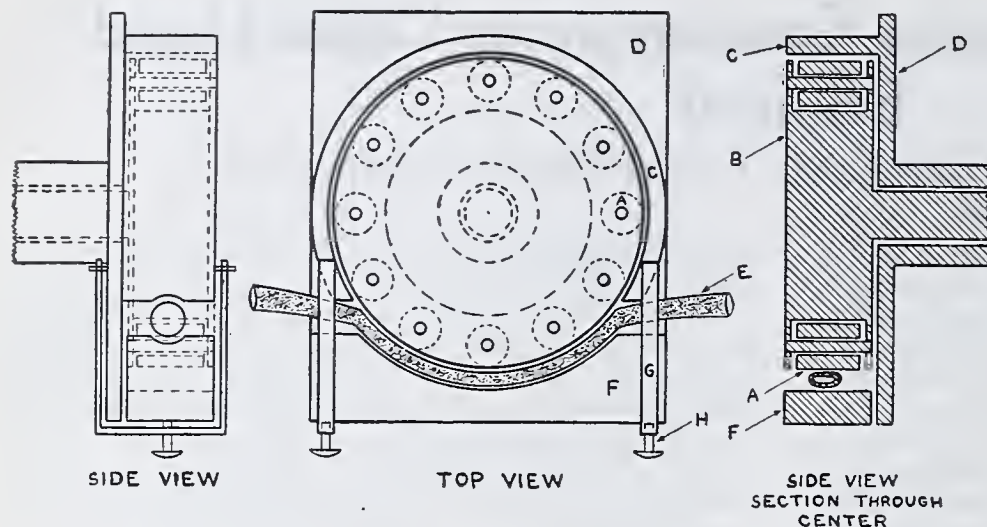


Figure 2. Circulating Pump

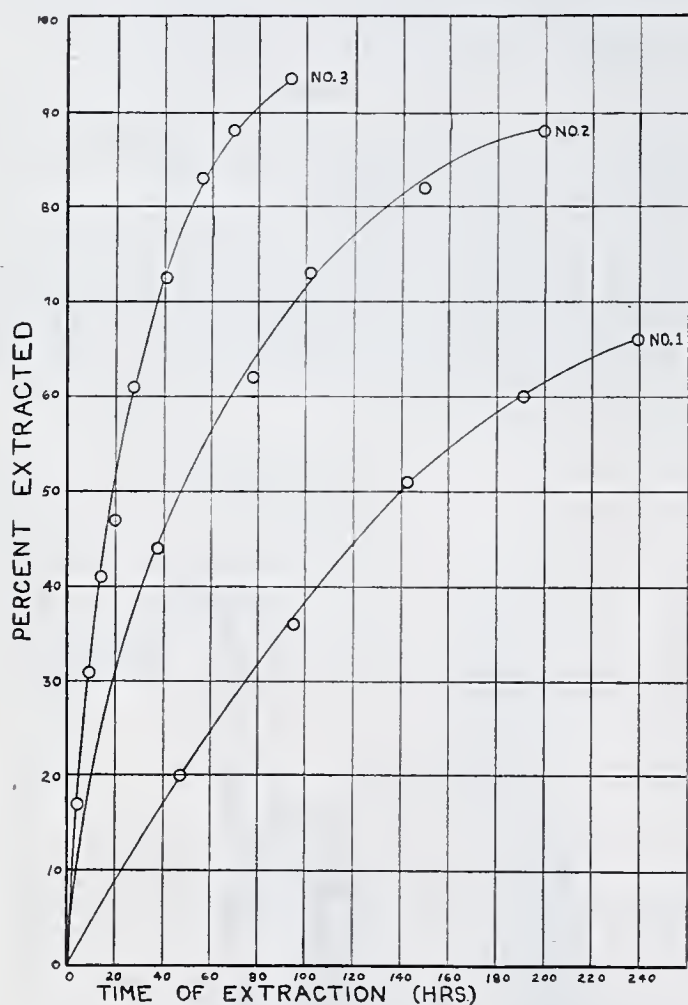


Figure 3. Extraction Rates for Three Extractors

The stirrer was driven at 700 r.p.m. Extractor 3 was the one described in this paper (Figure 1). The solvent flow rates were nearly the same for extractors 2 and 3, but for No. 1 the rate was somewhat less, being limited by the resistance of the diffuser plate.

The time-recovery data for the three extractors for 4 liters of mash concentrate containing 15% of 2,3-butylene glycol extracted with diethyl ether are plotted in Figure 3. The extractions were not carried to completion, since it is more efficient to reconcentrate the mash after the butylene glycol content has been considerably reduced. It is evident from Figure 3 that the extraction rate for the extractor described in this paper is greater than that of either of the other two extractors. The periods of

time required to remove 50% of the butylene glycol present for extractors 1, 2, and 3 were 140, 48, and 19 hours, respectively.

The extraction unit as described has been used in these laboratories for a considerable period of time for extracting 2,3-butylene glycol or acetylmethylcarbinol from fermentation mash, using diethyl ether as the solvent. It could be used equally well for extracting other liquids and with other immiscible solvents. No change in arrangement of the equipment would be necessary for other solvents of less density than the liquid to be extracted, and modifications could easily be made to enable the use of solvents of density greater than the liquid to be extracted.

In the latter case the layer of liquid to be extracted is at the top and the solvent fills the lower portion of the column. The pump draws the liquid from the top and introduces it into the solvent through a jet extending up from the bottom of the column, the droplets of liquid rising through the heavier solvent. The solvent from the condenser flows by gravity through a vertical tube extending from the top of the column down into the solvent layer. The solvent flows from the bottom of the column through a U-tube and a vertical leg of tubing connected horizontally at the top with the solvent boiling flask. The length of the vertical leg must be adjusted according to the density of the materials employed, so as to maintain the proper solvent level, and the horizontal tube connecting with the boiling flask must be of relatively large diameter to prevent siphoning action. The authors have successfully tested this arrangement, using chloroform as the solvent to extract an aqueous solution.

In setting up the countercurrent liquid-liquid extractor for any particular purpose the dimensions of the extraction column and the sizes of the flasks employed could of course be altered. The rate of extraction is naturally associated with the rate of recirculation of the liquids. If a smaller volume of liquid were extracted employing the same rate of circulation, the extraction would be completed more quickly, and vice versa.

#### ACKNOWLEDGMENT

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## Fifteen-Year Collective Index for Analytical Edition

A fifteen-year collective index of the ANALYTICAL EDITION of INDUSTRIAL AND ENGINEERING CHEMISTRY, complete through 1943, is being prepared by Charles L. Bernier, associate editor of *Chemical Abstracts*, with the expectation of being able to issue it early in 1945, as a pamphlet of the same page size as regular issues of the ANALYTICAL EDITION, if sufficient paper for printing can be obtained.

Both subject and author indexes are to be included, following in general the practice of *Chemical Abstracts*, but with certain variations suggested by the special nature of this analytical index.

Present plans contemplate furnishing copies of the index at a nominal price to any subscriber to the ANALYTICAL EDITION who places his order before publication, and selling copies after that time at a somewhat higher price. At present no definite price can be quoted, as it will depend somewhat upon the number of copies printed. It will be very helpful if those wishing to receive the index will notify Walter J. Murphy, Editor, 1155 Sixteenth St., N. W., Washington 6, D. C., preferably prior to October 1.



# A New Type of Microburet

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**HYBBINETTE** and Benedetti-Pichler (1, 2) have described a microburet in which the delivery of liquid is controlled by surface forces. This buret is accurate and extremely simple in construction and operation, but is not applicable to all types of titrations. It fails, for example, in a microdetermination of the saponification number of a fat or oil. When the tip is removed from the liquid at the phenolphthalein end point, the flow of hydrochloric acid from the buret continues, probably because the presence of alcohol vapor above the liquid lowers the interfacial tension between the hydrochloric acid and the gaseous phase.

The buret described in this paper retains the advantages of the buret of Hybbinette and Benedetti-Pichler and is applicable to titrations in alcoholic solutions.

## APPARATUS

A Pyrex capillary with an internal diameter of 1.3 mm. was constricted at one end to form a tip with an internal diameter of 0.2 mm., and was bent at a right angle 7 cm. from the tip end. The longer leg (65 cm.) was attached by means of Hoffman lamps to a stout meter stick that had been sawed at the 60-cm. mark and was held in a horizontal position in an iron support by Fisher Castaloy clamps. As the clamps covered the readings at each end of the rule, the usable portion of the scale extended from the 2-cm. to the 58-cm. mark. The capacity between these marks was 0.72 ml.

The buret was cleaned by tilting it so that the wide end was lower than the tip, siphoning through it cleaning mixture, then water and drying by a stream of filtered air. It was filled for use by sucking the desired solution in through the tip. A piece of rubber tubing was attached to the wide end for this purpose.

A serious drainage error is incurred if the meniscus in a horizontal capillary buret moves faster than about 15 mm. per minute (1). Because of the comparatively wide and long tip, the meniscus in this buret moved at the rate of 90 mm. per minute. This velocity was decreased to 12 mm. per minute by the insertion of a piece of very fine thermometer capillary, 1 cm. long, inside the rubber tubing just next to the end of the buret.

The hydrostatic head in this buret is too great to be supported by the surface tension at the tip. Therefore the titrations were performed as follows:

A weighing bottle, 2 cm. in diameter and 5 cm. in height, served as the titration vessel. About 2 ml. of mercury and the solution to be titrated, usually about 0.5 ml., were put in the weighing bottle. Next, the buret was filled beyond the 2-ml. mark with the desired solution, which was allowed to drip into a waste beaker until the meniscus exactly reached the 2.00-ml. mark. At that instant, the pendant drop was removed by wiping with Kleenex tissue. Then the titration vessel was placed so that the tip of the buret was immersed in the solution to be titrated. When the end point was reached, the titration vessel was raised a few millimeters so that the tip was beneath the surface of the mercury. No efflux from the buret occurred under these conditions. The delivery of liquid could be controlled within 0.2 mm. by lowering or raising the weighing bottle.

A magnifying glass was used to read the buret.

The titration stand, illustrated in Figure 1, is very convenient for accurate control of the buret.

Two boards, 2 × 9 × 20 cm., are hinged together. A block attached to the lower board opposite the hinge supports it in an oblique position. Another block attached to the upper surface of the lower board holds between the boards a piece of rubber tubing, of such size that the upper board is supported in an almost horizontal position. The buret is so placed that the tip dips into the mercury when the weighing bottle is placed on the titration stand. A slight downward force on the upper board disturbs the rubber and moves the board so that the tip of the buret is in the solution to be titrated. Then the liquid flows from the buret. Upon removal of the downward force, the board springs back to its original position, sealing the tip with mercury.

## CALIBRATION

**METHOD 1.** A thread of mercury, 3 to 5 cm. long, was sucked into the dry buret and, by means of gentle suction or pressure, was moved so that the left end was exactly at 2.00 cm. The position of the right end was then read. The thread was moved so that the left end stood at the point previously occupied by the right end, and the position of the right end was again read. This operation was continued until the thread reached the right end of the rule. The mercury was then expelled and weighed. Three calibrations were performed by this method.

**METHOD 2.** The buret was rotated about its axis, until the tip formed an angle of about 15° with the horizontal, and was then filled with mercury. This mercury was delivered in increments of about 0.4 gram into a weighing bottle and weighed, the position of the meniscus being read after each delivery. Two calibrations were performed by this method.

All five calibrations agreed within a mean deviation of  $\pm 0.02$  cm. or better for the corrections to the linear scale. There was a discrepancy, however, in the average volume of mercury delivered per centimeter caused by neglect of the meniscus error in method 1. For method 1, the results were 0.013000, 0.013011, and 0.012995 ml. per cm.; for method 2, 0.013025 and 0.013021 ml. per cm.

## TITRATIONS

In order to test the buret, three different types of titrations were performed.

**SODIUM CARBONATE WITH HYDROCHLORIC ACID.** A solution of sodium carbonate was prepared to be 0.46589 weight-normal, and 30- to 50-gram portions were weighed and titrated with 0.5 *N* hydrochloric acid in a calibrated 50-ml. buret. Two drops of 0.003 *M* methyl orange were used as indicator. A buffer was prepared with 20 millimoles of sodium acetate, 113 millimoles of acetic acid, and 2 drops of 0.003 *M* methyl orange in 80 ml. The end point was taken as the reading when the titrated solution had the same color as the buffer. Four such titrations were run.

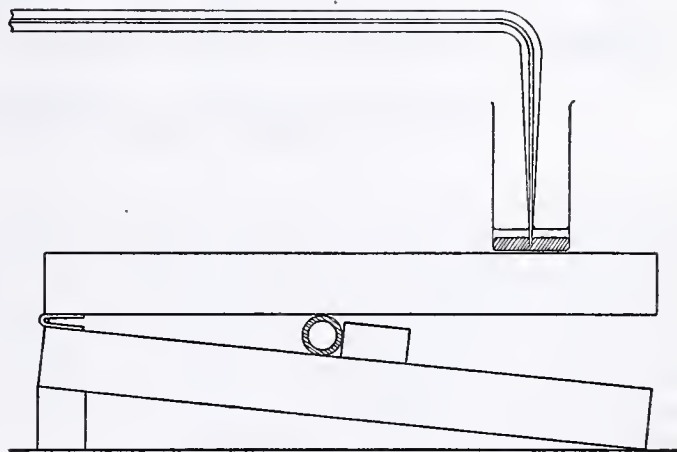


Figure 1. Titration Stand

For the microtitration, 0.5- to 0.7-gram portions of the sodium carbonate solution were weighed. One drop of 0.001 *M* methyl orange was added to each. A comparison buffer was used containing 0.30 millimole of sodium acetate, 1.7 millimoles of acetic acid, and one drop of 0.001 *M* methyl orange in 1.2 ml. Four such titrations were run.

**BENZOIC ACID WITH SODIUM HYDROXIDE.** Twenty drops of 0.03 *M* phenolphthalein, 4.9028 grams of pure benzoic acid, and approximately 200 ml. of neutral 95% ethanol were mixed. The resultant solution weighed 172.87 grams and was therefore 0.23224 weight-normal with benzoic acid. Three 16- to 19-gram portions of this solution were weighed and titrated with 0.1 *N* sodium hydroxide in a 50-ml. buret. Three 0.27- to 0.29-gram portions were weighed and titrated with the same sodium hydroxide in the microburet.

**SODIUM CHLORIDE WITH SILVER NITRATE.** Similarly three macrotitrations and three microtitrations of 0.17623 weight-



Table I. Results of Titrations

Reagents	Macrotitration		Microtitration		$\frac{v}{L}$
	Mean $\frac{W}{v}$	Relative mean deviation	Mean $\frac{W}{L}$	Relative mean deviation	
	G./ml.	Parts per 1000	G./cm.	Parts per 1000	Ml./cm.
Na <sub>2</sub> CO <sub>3</sub> -HCl	1.1204	±0.4	0.014542	±1.0	0.012979
C <sub>6</sub> H <sub>5</sub> COOH-NaOH	0.4483	±0.2	0.005813	±1.0	0.012967
NaCl-AgNO <sub>3</sub>	0.5771	±0.3	0.007500	±1.2	0.012996

normal sodium chloride with 0.1 *N* silver nitrate were performed. The indicator for the macrotitrations was 5 drops of 0.0025 *M* dichlorofluorescein; for the microtitration, one drop of 0.0001 *M* solution of the same compound.

**FERROUS SULFATE WITH PERMANGANATE.** Attempts were made to use the microburet in a titration of ferrous sulfate with permanganate. The mercury was oxidized so rapidly that even a fleeting end point was never attained.

The results of the foregoing titrations are summarized in Table I. The values in the second column are the mean ratios of *W*, the weight of the standard solution taken, over *v*, the volume of reagent used in the macrotitration. The values in the fourth column are the mean ratios of *W* over *L*, the quantity of reagent used in the microtitration measured in centimeters and corrected as indicated by the calibration. The values in the last column were obtained by dividing the values in the fourth column by the respective values in the second column.

#### DISCUSSION

The values for the relative mean deviation of the microtitrations (Table I) indicate that the precision of this microburet is satisfactory. From the values of *v/L*, it may be concluded that

the volume of dilute aqueous solution delivered per centimeter is independent of the nature of the solute. The slight discrepancy for the silver nitrate is probably due to the fact that the end point of this titration was not so sharp as that of the others. The values of *v/L* obtained by titration are appreciably smaller than the value obtained by calibration with mercury. This is due to the fact that water wets glass, whereas mercury does not.

The dimensions cited for this microburet need not be followed closely for other burets of this type. A capillary of smaller bore can be used if a smaller capacity is desired. A shorter buret would be more convenient and would require less time for a titration, but would be less accurate.

The advantages of this buret in comparison with that of Hybbinette and Benedetti-Pichler are the following: It can be used in the titration of alcoholic solutions; the tip is coarser and hence less fragile; and the tip is longer and can accommodate taller titration vessels.

On the other hand, this buret fails in titrations with permanganate and probably with any other strong oxidizing agent. It is common with the buret of Hybbinette and Benedetti-Pichler, it requires a long time for a titration because of the small velocity of the meniscus.

#### ACKNOWLEDGMENTS

Suggestions from Robert Munch and Stephen H. Laning regarding the calibration are gratefully acknowledged.

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## Thiamine Content of Pharmaceuticals

### Comparative Study of Rat-Curative, Thiochrome, and Fermentation Methods

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With 29 pharmaceutical products, no marked differences were obtained by the rat-curative, thiochrome, and fermentation methods. The average for the thiochrome values was 95.4% of the rat-curative values, 83% of the samples being less for the thiochrome than for the rat-curative. The average for the fermentation values was 98.3% of the rat-curative values, 62% of the samples being less for the fermentation than for the rat-curative. The ranges of values were moderate and indicate that, for these products, any one of the three methods was satisfactory.

**T**HE importance of laboratory control of the vitamin content of foods and pharmaceuticals needs no emphasis. While great progress has been made in developing practical analytical methods, uncertainties of precision and of accuracy still beset the analyst. In this paper, a comparative study of the assay of one class of materials is presented. Results are shown for the thiamine determination of a number of pharmaceutical products, for which three methods, involving quite different principles and procedures, were used.

Considerable effort has been spent on the estimation of thiamine by several methods and by various modifications thereof. However, many of the publications describing these efforts do not bear on the present problem. This study is deliberately

limited to the use of the rat-curative bioassay (U. S. P. XII) the thiochrome and the fermentation assays. These have been previously used in comparative studies and the results were sufficiently encouraging to warrant their use in this investigation.

Hennessy and Cerecedo (5) reported fair agreement between the results of thiochrome and rat-growth assays on identical samples. Frey and Hennessy (4) summarized the results of comparative assays on cereal products by a group of collaborators, each of whom used one or more of the three assay procedures. Cole, Jones, and Christiansen (2) compared the results of thiochrome and biological assay on a limited group of pharmaceuticals without noticing serious disagreement. Conner and Straub (3) assayed a few cereals and one vegetable by both biological and thiochrome assay, with satisfying results. Lane, Johnson, and Williams (8) reported the thiochrome and biological assay results concordant except for cooked pork. The fermentation method was shown to give somewhat higher results on beef than the Hennessy procedure by Hinman, Halliday, and Brooks (7). Biological assays were reported by Brown, Hamm, and Harrison (1) to give significantly higher values than the thiochrome procedure.

#### EXPERIMENTAL

The methods of assay were used without any attempt to modify the procedures as described in the literature.



Table I. Thiamine Determinations

No.	Stated Content	Thiochrome	Fermentation		Rat-Curative
			Total	Corrected <sup>a</sup>	
1	220 U.S.P. units/g.	217	259	212	268
2	1.5 mg./capsule	1.60	1.72	1.63	1.66
3	1.0 mg./capsule	1.05	1.21	1.13	1.05
4	333 U.S.P. units/capsule	351	382	343	333
5	1.0 mg./capsule	1.02	1.25	1.08	1.17
6	1.5 mg./capsule	1.56	1.78	1.63	1.84
7	500 U.S.P. units/capsule	502	519	500	527
8	500 U.S.P. units/fluid oz.	528	614	563	585
9	500 U.S.P. units/capsule	569	591	562	580
10	1000 U.S.P. units/capsule	1000	1028	974	1180
11	50 U.S.P. units/tablet	60	74	64	59
12	50 U.S.P. units/tablet	55	65	59	59
13	330 U.S.P. units/g.	331	377	326	350
14	500 U.S.P. units/fluid oz.	550	683	662	584
15	125 U.S.P. units/tablet	117	136	113	123
16	1.5 mg./capsule	1.56	1.75	1.46	1.62
17	500 U.S.P. units/capsule	515	600	549	558
18	60 U.S.P. units/tablet	62	80	70	64
19	50 U.S.P. units/tablet	58	70	63	54
20	111 U.S.P. units/tablet	130	124	117	130
21	333 U.S.P. units/capsule	363	384	351	370
22	500 U.S.P. units/capsule	533	550	494	522
23	333 U.S.P. units/capsule	347	366	360	370
24	350 U.S.P. units/capsule	373	386	381	400
25	85 U.S.P. units/g.	90	111	92	87
26	333 U.S.P. units/capsule	343	373	371	346
27	50 U.S.P. units/tablet	61	73	68	63
28	1.5 mg./tablet	1.60	1.80	1.73	1.70
29	72 U.S.P. units/ml.	82	96	84	87

<sup>a</sup> For sulfite correction.

**RAT-CURATIVE BIOASSAY.** The method as described in the U. S. Pharmacopoeia XII was used. No difficulty was encountered in feeding a sufficient quantity of sample, since all products were of fairly high potency. The assay was carried out at a definite level in each individual case. To minimize errors due to extrapolation, a scheme of interpolation was employed. This was made possible by running a curative series in which 5, 6, and 7 micrograms of thiamine chloride were fed to groups of 10 polyneuritic rats each. It was found that the number of days in which 10 rats remained cured were: for 5 micrograms 82 days, for 6 micrograms 107 days, and for 7 micrograms 129 days. Since the unknown content did not differ from the assumed content by more than 21% in any case, the possible error due to interpolation could not be of significant magnitude.

**THIOCHROME ASSAY.** The method described by Hennessy and Cerecedo (5) as modified by Hennessy (6) for routine analysis was followed. The maximum ratio of extracting solvent to sample and the minimum quantity of thiamine in the final aliquot, consistent with the necessary precision, were used. The sensitivity of the fluorophotometer permitted the measurement of 0.5 microgram of thiamine with a sensitivity of 0.005 microgram, so that this level of vitamin was approximated in the final aliquot in all assays.

**FERMENTATION ASSAY.** The method as described by Schultz, Kin, and Frey (9) was followed, except that the larger apparatus described in their earlier papers was used. The sulfite correction was made in every determination.

No unusual precautions were used in the assays; the customary care necessary in the analyses was observed. Duplicate determinations were made. If marked differences (greater than 10%) appeared in such duplicates, as happened occasionally, the assay was repeated.

Twenty-nine pharmaceutical products, made up in various ways by different manufacturers and containing different ingredients, were tested. Included were capsules containing a number of the synthetic vitamins, fish liver oil concentrates, yeasts, liver and iron compounds, yeast and iron compounds, tablets containing synthetic vitamins, yeast concentrates, Solvitain and iron compounds, liquid preparations containing yeast extract, yeast concentrate, and malt extract.

In the following tables the products are listed by number and a statement is given as to their composition. Attention is focused entirely on the thiamine content. The values in the "stated" column refer to the thiamine content of the products as stated by the manufacturer or on the label. These are generally 5 to 10% lower than the actual content because of the common practice of adding a slight excess to ensure adequate shelf life. The experimental values as given under the thiochrome, fermentation, and rat-curative headings were those found in the assays. Table I summarizes these values.

The order of the products given in Table I follows no set plan. If arranged by type of product, thiamine content, etc., no regularities were apparent. The products tested were sixteen capsules, seven tablets, none chocolate-coated, five liquids, and one powder.

In order to evaluate the significance of the experimental results, it is convenient to recalculate to a common basis. This is done in Tables II and III, in which the results of the rat-curative and thiochrome assays are placed at 100%, respectively, and the other data recalculated to this new basis.

These tables are summarized in Table IV to show the comparative ranges of the results obtained by the different methods of assay.

A number of relations are evident in these groupings. With the curative at 100%, the thiochrome results showed 24 products below 100%, 5 of them below 90%. The fermentation results showed 19 products below 100%, also with 5 below 90%. When the thiochrome results were set at 100%, the fermentation results showed 18 products above 100%. In general the two biological methods tend to give higher thiamine content than

Table II. Relative Thiamine Content

(Based on rat-curative = 100 for each product)

No.	Thiochrome	Fermentation	Stated
1	81	79	82
2	96	98	90
3	100	108	95
4	105	103	100
5	87	92	85
6	85	89	81
7	95	95	95
8	90	96	85
9	98	97	86
10	85	83	85
11	102	108	85
12	93	100	85
13	95	93	94
14	94	113	86
15	95	92	102
16	96	90	93
17	92	98	89
18	97	109	94
19	107	117	93
20	100	90	85
21	98	95	90
22	102	95	96
23	94	97	90
24	93	95	88
25	103	106	98
26	99	107	96
27	97	108	79
28	94	102	88
29	94	97	83
Av.	95.4	98.3	89.6

Table III. Relative Thiamine Content

(Based on thiochrome = 100 for each product)

No.	Fermentation	Rat-Curative	Stated
1	98	124	101
2	102	104	94
3	108	100	95
4	98	95	95
5	106	115	98
6	105	118	96
7	100	105	100
8	107	111	95
9	99	102	88
10	97	118	100
11	107	98	83
12	107	107	91
13	99	106	100
14	120	106	91
15	97	105	107
16	94	104	96
17	107	108	97
18	113	103	97
19	109	93	86
20	90	100	86
21	95	102	90
22	93	98	94
23	104	107	97
24	102	107	94
25	102	97	94
26	108	101	97
27	112	103	82
28	108	106	94
29	102	106	88
Av.	102.9	105.1	94.0



Table IV. Comparison of Results by Three Methods of Assay

Percentage ranges	<80	80-85	86-90	91-95	96-100	101-105	106-110	111-115	116-120
No. of thiochrome vs. curative (100%)	0	3	2	10	9	4	1	0	0
No. of fermentation vs. curative (100%)	1	1	3	7	7	2	6	1	1
No. of fermentation vs. thiochrome (100%)	0	0	1	3	7	6	9	2	1
Thiochrome vs. curative (100%) -19 to +7	Per Cent Extremes Fermentation vs. curative (100%) -21 to +17								
	Fermentation vs. thiochrome (100%) -10 to +20								

the chemical procedure. The agreement between the fermentation and thiochrome data (25 between 91 and 110%) is better than between the thiochrome and curative (24 between 91 and 110%) because of the nearly even distribution in the former comparison on both sides of 100%. It is also better than the agreement between the fermentation and the curative results

(22 between 91 and 110%) because of a greater grouping of the former in the range 96 to 105%—13 as compared to 9.

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## Microapparatus for Purification of Solids

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IN THE recrystallization of small amounts of organic compounds under certain conditions it is difficult to use conventional apparatus. Mechanical losses, such as those incurred in transfer to a suction filter, and contamination by evaporation of solvent from the crystalline mass, became major problems in certain cases. For example, in a recrystallization from a small amount of solvent, where there is little filtrate available, washing crystals on the filter or using mother liquor as a wash liquid is unsatisfactory. The clean filtration of hygroscopic or sticky crystals, or those suspended in a thick sirup, is often difficult with the usual suction filter.

The apparatus shown in Figure 1, a modification of the Skau (1) tube, has found application in this laboratory to problems of this type. The modifications consist of the introduction of a sintered-glass plate as the filter medium and a design which minimizes losses and eliminates the necessity of transferring the crystals to another vessel for further treatment.

The apparatus is used for recrystallization as follows: The material to be recrystallized is dissolved in a hot solvent in the small flask, A (Figure 1); for this purpose it is often convenient to attach A to a reflux condenser by means of its standard-taper ground joint. After cooling to crystallization the filter section and flask B are assembled as shown in Figure 1, inverted, and centrifuged. It is important to keep the ground surfaces of the joints free of any solid material to prevent the joint from sticking. The lower flask (now flask B) containing the filtrate is then removed by grasping the rim, c, if necessary with the aid of a wooden wrench, and loosening the joint by a firm twisting motion of B. No difficulty is experienced at this point unless the joint is put together while dirty or the tube is centrifuged at too high a speed. In the event that the joint becomes stuck it may readily be taken apart by use of V-shaped wooden wedges f (Figure 2), driven as shown between flanges c and d. This method of loosening ground joints has been successful in almost all cases except where serious etching of the glass by alkali has occurred. (The author is indebted to F. P. Noble, glass blower in this laboratory, for this valuable suggestion.)

The crystals are now shaken back into A and, if further recrystallizations are desired, washed down with the solvent. This washing is easily accomplished without removing the filter from A by adding the solvent above the filter disk, b, and cooling A. Contraction of the gas in the chamber draws the solvent through the filter into the flask, washing down the sides at the same time. Centrifuging is seldom necessary at this point. Any number of recrystallizations can be carried out without opening flask A to

the air (diffusion through disk b is slow) or incurring a loss due to transfer of solid from one vessel to another.

The dimensions shown are chosen so that the tube will fit the standard 50-ml. carriers for a size 1 centrifuge (International)

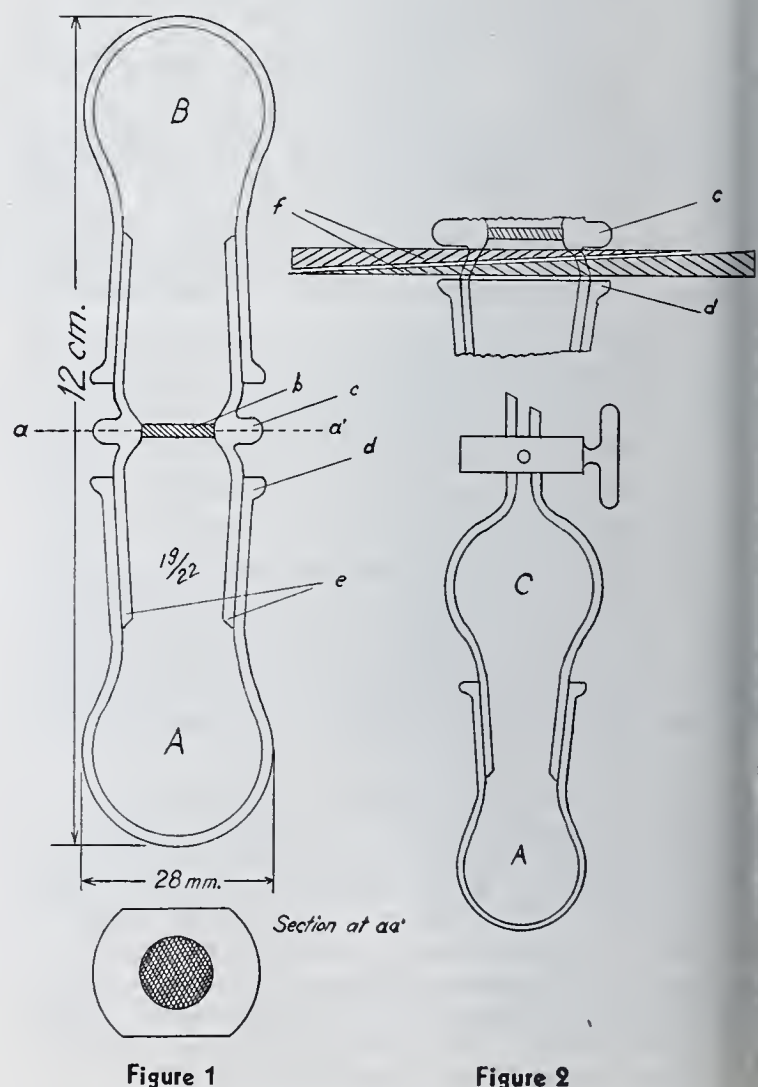


Figure 1

Figure 2



and a variety of shapes and sizes of flasks may be used. The use of larger sintered-glass disks, *b*, than 10 mm. is attended with some risk of breakage, but extensive experiments on this point have not been carried out. The male 19/22 ground joints have been beveled on the inside, *e*, to minimize retention of material at the ends of this joint, otherwise there may be losses due to filtrate being driven through the ground joint while in the centrifuge. The sintered plate, *b*, of medium porosity (Jena G-3 or Pyrex C) has been found most useful for general purposes, but finer filters can be used successfully.

Tubes of the size shown have been in use in this laboratory for several years without a single breakage in the centrifuge at speeds up to 1800 r.p.m. This speed has been found adequate to drain the liquor thoroughly from almost any type of crystals in a few minutes. With nicely formed crystals in the usual solvents (alcohol, ether, etc.) filtration is complete in a few seconds and the crystals are found free enough from solvent to be shaken readily from the filter into *A* by gently tapping the flask. Sticking of the ground joints at these speeds practically never necessitates the use of wedges and the apparatus is readily taken apart by hand as described above. Mechanical distortion of organic crystals in centrifuging has not been observed under the conditions described.

It has been found convenient to combine with the above vessels the bulb *c* (Figure 2) to facilitate extraction with immiscible solvents.

The two phases—for example, a diluted reaction mixture—are placed in *A* and shaken to equilibrium as with the usual separatory funnel, releasing pressure through the stopcock if necessary. The apparatus is then inverted, *A* removed, and the lower phase drawn out of *C* through the stopcock. *A* may now be replaced and the assembly righted again for further treatment. The capacity of *C*, including the joint, must be greater than the bulb of *A*. A more convenient and cleaner separation of phases is possible on a small scale with this apparatus than with a pipet such as is commonly used for this purpose in micro work.

Other pieces of equipment fitted with 19/22 joints are useful in preparative work on this scale—for example, still heads, reflux condensers, etc. These standard types of apparatus, which are readily attached by means of the standard-taper joints, do not require special description and are varied to suit individual problems.

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# Microtechnique of Qualitative Organic Analysis

## Identification of Organic Acids by the Partition Method

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DEVELOPING the microtechnique of qualitative analysis of the organic acids the authors found that the adaptation to a micro scale of the method of Duclaux for identifying the volatile members of the fatty acid series presented serious difficulties. The method requires careful measurement of the volumes of sample and distillates and control of the rate of distillation. Although the use of very dilute solutions might permit fulfillment of the first requirement, the Duclaux procedure calls for at least 0.5 *M* solutions of the acids. A modification of distillation apparatus of the Craig (3) type could possibly be used for collecting distillates of small known volumes, but control of the rate of distillation of 1 or 2 ml. of liquids is very difficult. On the other hand, the extraction procedures of Behrens (1) and Werkman (5, 6) not only overcome these difficulties but also seem to possess a number of advantages over the Duclaux method even on a macro scale. Some of these advantages which can also be realized when working with microsamples are: (1) The method is not limited to the volatile acids; (2) a great saving in time may be effected; (3) the apparatus and procedure are much simpler; (4) the "constants" obtained are not affected by as many factors; and (5) the method is more accurate and reliable.

In principle the method consists of extracting an aqueous solution of the acid of known concentration with an organic solvent such as diethyl or isopropyl ether. After the extraction the concentration of the acid in the aqueous layer is again determined and the ratio of the concentrations before and after extraction is the "partition constant". It has been found that this constant is characteristic of the acid.

The procedure given by Werkman and co-workers

was followed in general in developing the microprocedure, but changes were made whenever the microtechnique permitted simplification or shortening of the work. At first the authors attempted to use the refractive index of the solutions as a measure of the concentrations of the acids. This proved satisfactory when using higher concentrations of formic, acetic, propionic, and butyric acids. However, the applicability of this method of measurement was limited by the solubility of the acid and the degree to which the particular acid is extracted. Obviously, the less soluble the acid, the more dilute its solution will be and the nearer the index of refraction of the latter to that of pure water. Also, the more the acid is extracted from the aqueous solution, the more dilute and the more like pure water the latter will become. However, the results obtained with the more soluble acids show that the method has possibilities, especially where nonvolatile acids such as citric or lactic are concerned.

Although Werkman recommends and uses 0.03 to 0.1 *N* aqueous solutions of the acids, the authors of the present paper used 0.3 *N* solutions. This concentration permitted the employment of volumes large enough to be handled easily in micropipets and burets, but it was sufficiently high to avoid most of the difficulties met with in the titration of very dilute solutions (4). The ratio of ether to water was decreased from that given by Werkman in order to obtain a greater difference between the values of the constants for the various acids.

In the first experiments carried out, the volumes of the acids and extractant were measured out in capillary pipets. The results were not found to be very precise. A Benedetti-Pichler-Hybbinette buret (2) was therefore used both for measuring out the volumes of the acids and ether and for titrating the original and extracted solutions.

#### METHOD

An extraction tube is prepared by sealing one end of a glass tube of 4-mm. bore and 150-mm. length. The 0.3 *N* acid solu-

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tion is introduced into this tube from the buret, a length of 120 mm. of the solution being taken, followed by 150 mm. of ethyl ether. The open end of the tube is sealed and the contents are centrifuged back and forth several times. The tube is then cut open close to the surface of the ether. A capillary pipet with a mark at the 150 cu. mm. point (this need not be exactly 150 cu. mm. but the volume should be less than the total volume of the original acid solution) is prepared as shown in the figure. The capillary portion of the pipet is about 150 mm. long and 1.5 mm. in bore. The wider portion is about 60 mm. long and 5 to 6 mm. in diameter. This is dipped down into the aqueous layer in the extraction tube, the upper end of the pipet being held closed with the finger. Since even with this precaution some of the ether layer will enter the pipet, a bubble or two of air is carefully blown through the pipet to displace any ether solution. Then the required volume of the aqueous layer is drawn into the pipet. This sample is transferred to a microbeaker and there titrated, using 0.3 N sodium hydroxide and phenolphthalein as indicator. Using the same pipet, an equal volume of the original acid solution is titrated in the same way. The ratio

$$\frac{\text{Volume of alkali used for extracted sample}}{\text{Volume of alkali used for original sample}} \times 100$$

is the partition constant.

The following values were obtained using the ratio of acid to ether 12 to 15 and a concentration of original acid about 0.3 N.

Formic acid	64.3
Acetic acid	48.7
Propionic acid	33.7
Butyric acid	14.5
n-Valeric acid	5.0

The values of the respective constants are so far apart that the acid can be identified without difficulty, even if small errors are made during the determination.

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## Staining Rubber in Ground or Milled Plant Tissues

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Following suitable pretreatment of samples, differential staining with Sudan IV and iodine green has proved a useful and relatively rapid aid in the microscopic study of rubber in ground guayule tissues, mounts in many cases being ready for examination within 90 minutes after starting the schedule.

**A**CCCESSORY to a comprehensive study of guayule production now under way by the United States Department of Agriculture is an analysis of the rubber content of plants of various strains and ages grown under diverse environmental conditions. This analysis is currently made by chemical extraction of the rubber from finely ground tissues by a method based on that described by Spence and Caldwell (9). This method consists of a number of distinct steps and it is part of the program to test possible variants of these steps. As a check on the effectiveness of such tentative modifications, the ground samples are examined microscopically, especially the spent charges remaining after extraction.

#### RUBBER-STAINING DYES

In the microscopic inspection of tissues it is common practice to use various dyes for staining diverse plant substances, sometimes singly, sometimes in combination (3, pp. 9-12, 167-174). For staining rubber in plant sections, Lloyd (6) used alkanet, while Hall and Goodspeed (4, p. 212) and Artschwager (2) employed Sudan III. Other dyes which stain guayule sections in patterns similar to those of Sudan III are Sudan IV, Sudan black B, and Calco oil blue NA. Unfortunately, these dyes are not specific for rubber but also stain various other materials, such as resins, oils, fats, suberin, and cutin (3, p. 210; 4, p. 213; 5, p. 63; 6; 8, pp. 47, 48, 58; 10). Steps must accordingly be taken to eliminate these substances from the sample or to learn to recognize them by some distinctive character of form, color, or location. An experienced microscopist or technician might be expected to recognize the various tissues and substances in plants with which he is working, often without any staining at all (4,

p. 212; 6). For many workers, however, staining and counterstaining will prove an exceedingly useful aid in studying plant material. It is for this group that these notes are intended.

Suberin can be decomposed by treatment with potassium hydroxide in ethyl alcohol (8, p. 47), after which the cork cell walls no longer take up the rubber-staining dye. Any fats that may be present will likewise be saponified by the potassium hydroxide. For getting rid of resins and oils acetone or ethyl alcohol may be used (4, pp. 212, 214; 6). Pigments can be bleached out by means of oxidizing agents.

Needless to say, the microscopic examination of ground samples is a far different matter from that of ordinary sections. In the ground material the various structural elements are mostly much displaced from their original relative locations, many of the fragments are thicker than the usual run of sections, and the pieces are sometimes piled one on top of another. The face exposed under the cover glass of a microscopic mount is usually longitudinal, cross-sectional exposures being uncommon. Before extraction, stained rubber agglomerates are likely to be fairly plentiful, especially in the case of plants of high rubber content.

#### STAINING SCHEDULE

The choice of dye is partly a matter of personal preference. Sudan III colors rubber somewhat scarlet, while Sudan IV gives more of a crimson cast. Sudan black B gives an indigo or dark blue, approaching black, and Calco oil blue NA a much brighter blue. Addicott (1) has recently described a combination dye resulting in a blue staining of the rubber with red coloration of lignified and suberized tissues. In working with ground material of guayule and other plants, the author found that Sudan IV combined with either iodine green or methyl green yields mounts showing striking contrast between the crimson of the stained rubber and the blue-green coloration of the woody tissues and cork cells. After considerable testing of various schedules, he tentatively settled upon the following as giving very satisfactory differential staining to the ground tissues studied, besides being comparatively fast in use:



Soak in potassium hydroxide solution	15 minutes
Add bleaching solution and soak	10 minutes
Rinse with water	
Stain with a mixture of Sudan IV and iodine green (or methyl green) solutions	0.5 hour
Rinse with water	
Mount in glucose sirup	

## Solutions used

1. Potassium hydroxide	10 grams
95% ethyl alcohol	100 ml.
2. A commercial bleaching agent (Clorox) contain- ing 5.25% sodium hypochlorite and 94.75% inert ingredients (according to analysis pub- lished on the label)	
3. Sudan IV (dye content 86%)	0.095 gram
Acetone	47.5 ml.
70% ethyl alcohol	47.5 ml.
4. Iodine green	0.095 gram
Acetone	47.5 ml.
70% ethyl alcohol	47.5 ml.
5. Methyl green (dye content 60%)	0.095 gram
Acetone	47.5 ml.
70% ethyl alcohol	47.5 ml.
6. A commercial table sirup [Karo (crystal white)] containing, according to the label, "corn sirup, sugar, salt, and vanilla"	

The sample to be stained is placed in a small shell vial to cover bottom to a depth of about 3 mm. To this is added about 1 l. of the alkali solution. Sample and solution are thoroughly mixed, care being taken to wet all particles. At the end of the saponification treatment approximately 2 ml. of the bleaching solution are added to the vial, without removing the alkali, and the preparation is thoroughly stirred as before. The sample is then washed out into a cone of tough filter paper and rinsed with several funnels of water. The sample mass is returned to its vial, dye solutions are added, about 0.5 ml. of each, and the preparation is once more thoroughly stirred. Good differential staining takes place in 30 minutes at room temperature. After the sample is washed out into a Syracuse watch glass, the water is drained off by capillary action of a small folded piece of bibulous paper, the dish is refilled, and the water again drained out. The stained sample, transferred to a microscope slide, is collected into a small pile and the excess moisture is drained away with a bit of bibulous paper. Mounting sirup is added, about 2 to 4 drops, and thoroughly mixed with the sample. With application of a cover glass the preparation is ready for examination.

## DISCUSSION

With the above-outlined schedule, rubber is stained crimson, lignified tissues and cork cells bluish green or blue-green. If suberin is not removed prior to staining, the cork cells stain purple or dark crimson or sometimes partly red and partly green. Although they can with practice be recognized by their distinctive structure, the purple or crimson color is not always clearly distinguishable from the stained rubber and inclusion of the alkali treatment is accordingly desirable. While Rawlins (8, p. 47) specifies boiling alcoholic solution of potassium hydroxide for removal of suberin and Miller (7) states that suberin is soluble in warm alkali, the present author has found the alcoholic solution equally effective at room temperature in removing from the cork cell walls of guayule, mariola (*Parthenium incanum* HBK), and yote brush (*Baccharis pilularis* DC), the material stainable by Sudan III, Sudan IV, Sudan black B, and Calco oil blue NA. Cold aqueous solution does not have this effect nor does 95% ethyl alcohol alone. With other plant species than those with which the author has worked the situation might be different. Each worker must, of course, study his own material.

Although a short treatment with the bleaching solution does not completely remove pigment from the larger tissue fragments, this is of relatively little significance when using Sudan IV as the rubber stain, since in guayule the residual color is a yellow green which is fully distinguishable from the stained rubber. Entire omission of the bleaching solution results in poorer staining with Sudan IV and iodine green. If both saponification and bleaching

treatments are omitted, the Sudan IV-iodine green mixture apparently fails to color rubber occurring in very small amounts, even with a 5-hour treatment. As far as the final staining is concerned, it makes little, if any, difference whether the bleaching agent is used before or after the saponifying solution, but the sample is easier to handle when the alcoholic potassium hydroxide solution precedes the bleaching solution.

In this schedule cutin, when present, stains a light pink or lavender color not readily confused with the crimson of the rubber. Leaf veins are dull light green or blue-green.

The question could be raised as to whether the stained agglomerates in unextracted ground samples might not be resin rather than rubber. The texture of these masses, however, is very definitely tough and elastic, indicating that they are at least mainly of rubber. Furthermore, in view of the fact mentioned above that resins and oils are soluble in acetone and in ethyl alcohol, it seems probable that these plant substances are at least in part removed by the solvents used for the alkali and dye solutions.

Another application of this staining schedule is in studying factory bagasse as a check on the completeness of rubber extraction in commercial milling.

The sirup used in this technique, which was suggested by Johansen (5, p. 24), sets rather slowly, and slides must accordingly be kept flat for some days or weeks after specimens are mounted. In preparations held for 2 months at room temperature the cover glass can be removed only with considerable difficulty, while even those only a month old are fairly well set. Although this sirup mixes freely with water, an excess of water under the cover glass is undesirable because it tends to flow to the edges, subsequently drying out and leaving ragged spaces on the border of the preparation. No mold has developed on any of the author's slides using this mounting medium, the oldest prepared 13 months ago.

It is the author's practice to mix the two dye solutions together at the time of adding to the sample, but they may be combined as much as a week in advance without affecting the final stain. Premixed dyes 4 weeks old gave differential staining, but much less brilliant than the fresher ones. There seems no change in color of the Sudan IV and iodine green staining in preparations even as much as 9 months old.

While the time required for rinsing varies considerably with the material, for many samples mounted slides will be ready for microscopic examination within 90 minutes from the beginning of the schedule.

## ACKNOWLEDGMENT

The author is indebted to Hamilton P. Traub for the suggestion that Sudan IV and Sudan black B might prove to be rubber-staining dyes.

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# A.S.T.M. Attracts Large Attendance of Analytical Chemists

L. T. HALLETT, Associate Editor

THE American Society for Testing Materials, holding its 47th Annual Meeting in New York, N. Y., at the Waldorf-Astoria June 26 to 30, attracted an ever-increasing number of analytical chemists to its deliberations. Widespread interest was shown in the sessions of Committees E2 and E3 and specially in the Symposium on Analytical Colorimetry and Photometry which was divided into two sections, one on instrumental applications and the other on specific chemical problems as exemplified by the paper by M. G. Mellon of Purdue on the subject "The Chemistry in Colorimetry".

Many analysts are familiar with Committee E3, formed in 1933, whose work is well known through its standard and tentative procedures. This committee now plans a special study and revision of a number of analytical procedures with a view to incorporating certain desirable modifications designed to employ more generally instruments such as the spectrophotometer, polarograph, etc., and adoption of newer techniques which have appeared in the literature since the standard procedures now in effect were originally accepted.

Committee E2, formed in 1936, dealing with spectrographic analysis, works closely with E3 because problems of preparation of samples and standardization require collaboration of these two groups.

The growing interest among analytical chemists in modern methods of analysis was amply demonstrated by the well-attended Symposium on Analytical Colorimetry and Photometry held on Wednesday, June 28.

Instruments and the physical principles underlying their use were discussed in the morning session, while in the afternoon specific methods of application and the chemical principles involved were presented. The complete program follows; the papers will appear in A.S.T.M. publications.

## I. INSTRUMENTAL SECTION

Fundamentals of Colorimetric Measurements. J. L. HAGUE, National Bureau of Standards.

Photocells in Colorimetry. R. H. MÜLLER, New York University.

Glass Photometric Filters. O. A. GAGE, Corning Glass Works.

Filter Photometers. A. E. RUEHLE, International Minerals & Chemical Corp.

Spectrophotometer Cells. S. E. Q. ASHLEY, General Electric Co.

Trends in Analytical Chemistry. B. L. CLARKE, Bell Telephone Laboratories.

Spectrophotometers. K. GIBSON, National Bureau of Standards.

Instrumental Errors. C. I. LUKE, Bell Telephone Laboratories.

Physical Basis of Light. R. J. KRYTER, Esterline-Angus Co.

## II. CHEMICAL SECTION

The Chemistry in Colorimetry. M. G. MELLON, Purdue University.

Bibliography on Colorimetric Methods. J. W. STILLMAN, E. I. du Pont de Nemours & Co.

Specific Methods for Aluminum-Base Alloys. J. J. STUMM, W. F. Jobbins, Inc.

Specific Methods for Magnesium-Base Alloys. V. A. STENGER, Dow Chemical Co.

Specific Methods for Other Nonferrous Alloys. C. ZICHSHKAU, American Smelting and Refining Co.

Specific Methods for Ferrous Alloys. A. THOMAS, American Rolling Mill Co.

Beverly Clarke, Chairman-Elect of the New York Section of the AMERICAN CHEMICAL SOCIETY and associated with the Bell Telephone Laboratories, welcomed those attending the symposium and expressed the hope that they would come back to the A.C.S. meeting in the fall, also to be held in New York September 10 to 15. The speaker emphasized the trend in analysis toward employment of physical instruments. The modern analyst must, therefore, be familiar, he pointed out, with simple electronics and the principles upon which these instruments are based, if he is to be highly successful.

The handling of analytical problems requiring instruments by those primarily trained in physics was not considered the best solution of the problem. The reason for this was brought out by Dr. Mellon's introductory remarks, when he emphasized that measurement of

color by modern instruments is rather simple but that to bring colorimetric procedure to a point where a constituent can be accurately and precisely determined is often difficult. The formation of color which will permit measurement of the concentration of the constituent in the presence of impurities is a problem where thorough training in chemistry and not in physics is the answer. The fact that calibration curves for certain colorimetric procedures do not obey Beer's law should not in itself condemn such a method.

K. Gibson, of the National Bureau of Standards, cautioned against the errors which are present in the measurement of the color of solutions which fluoresce, and pointed out that analysts should constantly keep in mind that instruments can give reproducible results that are very inaccurate. The speaker indicated the importance of using a high-grade spectrophotometer in setting up a new colorimetric method. He also pointed out that color-measuring instruments now manufactured require individual calibration when used in colorimetric determinations. Therefore it is impossible for a central laboratory to distribute standard curves which can be used universally.

R. J. Kryter, Esterline-Angus Co., demonstrated some of the properties of light as related to color measurement, showing why the eye is really a poor instrument for color analysis. The photographic plate is a slow and cumbersome means of measuring light and the photoelectric cell has largely taken its place in modern instruments.

Ralph Müller, on leave from New York University to M.I.T. for research on electronics, predicted that as the result of war research electronic devices released after the war will give the instrumental analysts many improvements not now thought possible. Lens systems made of plastics will be possible and the iconoscope will replace the photographic plate in certain light-measuring instruments.

The papers presented in the afternoon session demonstrated the use of filters and spectrophotometers in the analysis of alloys by colorimetric methods. Of particular interest was the use of spectrophotometers in analyzing complex color mixtures for more than one constituent.

## Analytical Edition Advisory Board Meets at Woods Hole

All members of the Advisory Board of the ANALYTICAL EDITION of INDUSTRIAL AND ENGINEERING CHEMISTRY met at Woods Hole, Mass., on Saturday, July 1, for one of its regular quarterly conferences.

Progress of the work on the Collective Index for the first fifteen volumes was reported, numerous questions of policy and procedure were discussed, and special features for coming issues were suggested. It was moved and seconded that the Chairman of the Division of Analytical and Micro Chemistry of the AMERICAN CHEMICAL SOCIETY be invited to participate unofficially in the deliberations of the board at future meetings.

## X-Ray Diffraction Unit

An x-ray diffraction unit developed by the North American Philips Co., 100 East 42nd St., New York, N. Y., has a four-windowed tube enclosed in a massive bronze housing. Diffraction patterns of four different specimens can be obtained simultaneously. A number of types of x-ray tubes can be provided, including targets of tungsten, molybdenum, cobalt, iron, chromium, and copper. Tubes can be changed quickly. Shockproof and rayproof, the unit utilizes full wave rectification, which permits higher output and longer life from the tube. Filament current supply is stabilized, control of kilovoltage and milliamperage is smooth and stepless, tube is protected automatically if water supply fails, equipment has start-stop pushbutton control, and provision is made for use of automatic exposure timer.



## Methods of Analysis for Anhydrous Hydrofluoric Acid

Procedures Recommended by Manufacturing Chemists' Association Committee (1944):  
W. B. Sherry, General Chemical Company, C. F. Swinehart, Harshaw Chemical Company,  
R. A. Dunphy, Kinetic Chemicals, Inc., and S. C. Ogburn, Chairman, Pennsylvania  
Salt Manufacturing Company

The Analytical Edition is privileged to present here procedures for anhydrous hydrofluoric acid recommended by the Manufacturing Chemists' Association Committee (1944). While these naturally include contributions from one of its members which have appeared in the ANALYTICAL EDITION [Swinehart and Flisik, 16, 419 (1944)], the methods as published here constitute the association committee's official report and hence are printed in full.

**A**NHYDROUS hydrofluoric acid is a colorless, fuming, corrosive liquid at relatively cool temperatures. Although it has a pressure of only about 0.5 pound gage at 22° C., it is shipped in pressure containers because its boiling point (19.4° C.) is often exceeded by the temperatures at which it is transported and used. Its freezing point is approximately -83° C., its vapor pressure is approximately 2.5 atmospheres at 50° C., and its density is 1.0 at 0° C. Its heat of vaporization is about 6000 calories per formula weight, and it has a low viscosity and surface tension. For all practical purposes, pipe sizes used in handling the liquid anhydrous hydrofluoric acid may be based on water data. As it is very hygroscopic, due care should be used to prevent moisture pickup in order to avoid errors in analysis, and precautions should be taken to prevent accumulation of water in apparatus, lines, or valves used.

Liquid anhydrous hydrofluoric acid attacks glass and most organic substances. It will react immediately with the skin upon contact, causing serious burns, and its fumes are irritating to the eyes and the mucous membrane. Care should be used to avoid contact with the liquid acid and its fumes, and workers should wear proper rubber-coated coat or apron, neoprene gloves, and face shields. Hoods with adequate ventilation are indicated for working with this acid in the laboratory; otherwise proper respirators should be worn. Water supply should be readily available for thorough and prolonged flushing in case of accidental contact with the skin.

Steel, Monel, copper, silver, and platinum are resistant to the acid, and may be used in the construction of equipment for handling it. Cast iron, or other metals containing silicon, should not be used with anhydrous hydrofluoric acid.

### GENERAL PRECAUTIONS

The Division of Industrial Hygiene, National Institute of Health, has issued specific recommendations for the treatment of hydrofluoric acid burns, prepared in consultation with medical and technical personnel of acid manufacturers (2, 3).

### SAMPLING PROCEDURES

The taking of the sample in many instances constitutes an important part of the analysis. Obviously the more closely the sample is representative of the material whose composition is

sought, the more nearly will the subsequent analysis reflect its true composition. It is fortunate from an analytical standpoint that anhydrous hydrofluoric acid is ordinarily in the form of a homogeneous liquid and the analysis is generally reported on the liquid phase. On the other hand, amounts of impurities present in the anhydrous hydrofluoric acid currently manufactured are of such a low order of magnitude that extreme care is necessary in order that the sample taken for analysis shall not be vitiated by the loss or addition of any of these impurities.

The principal impurities in anhydrous hydrofluoric acid are silicon tetrafluoride, sulfur dioxide, fluosulfonic acid, sulfuric acid, water, and metallic salts. The determination of the total acidity and the percentage of actual hydrofluoric acid in the sample are of fundamental importance.

Anhydrous hydrofluoric acid is now generally stored in steel tanks and shipped in steel tank cars, and it is transferred from the storage tank to the tank car, or vice versa, by two different methods—i.e., by blowing with compressed air (or other suitable gas), or by means of a pump. The use of a pump simplifies the taking of a representative sample from any storage tank, or tank car, if the discharge line from the pump is connected not only to the point where the acid is to be transferred but also back to the tank, or tank car, from which the pump is delivering acid—in other words, if the acid is thus recycled for a sufficient length of time, a sample properly drawn from a suitable connection on the pump line would be truly representative of the material in the tank, or tank car. Where the “blowing” method of transfer is used, a sufficient quantity of acid must first be blown through the lines to eliminate any impurities in the lines in order that the sample, when taken, may be identical with the material in the tank, or tank car.

**CYLINDERS FOR TAKING SAMPLES.** Cylinders with valves suitable for hydrofluoric acid make convenient containers for drawing samples of anhydrous hydrofluoric acid. These cylinders may then be transported to the laboratory where small amounts are taken out for analysis. Cylinders should not be filled to more than 85% of their water capacity by weight. (They should also be hydrostatically tested before being put in this service; a test pressure of at least 300 pounds per square inch is recommended.)

Cylinders usually used have water capacities of approximately 8.8 pounds or 120 pounds. It is essential that the tare weight and capacity be known for each cylinder. Cylinders should be weighed as they are filled and again when disconnected,







the hydrogen fluoride, while that in the top serves to trap any vapors formed through local concentration of heat.

The entire weight of ice must not greatly exceed 130 grams, as this amount when melted, plus about 40 grams of sample, will not allow the liquid level to rise above the outlet of the Saran tube. If the setup is exactly as described above, a clearance of about 2 inches is assured. (*Caution.* Never allow the Saran tube outlet to be submerged during the addition of the sample, for then a very rapid suck-back results, which would ruin the sample and may cause an explosion.)

With the sample cylinder in place, open the sample cylinder valve slightly (*Caution.* Rubber gloves should be worn.) and allow a few milliliters of the acid to flow out into a Monel waste beaker to sweep the outlet free from any condensed water that may be present. Place the sample weighing tube (without the rubber stopper) on the Harvard trip balance (as shown in Figure 2) and immediately couple the Saran tube to the cylinder adapter, tightening with a wrench. Balance with the rough weights and make certain that the Saran tube does not hinder the balance swing, then add 40 grams more weights. Carefully open the sample cylinder valve slightly, and adjust the flow of anhydrous hydrofluoric acid to about 10 grams per minute. As the acid strikes the ice, a sizzling sound may be faintly heard and through this guidance the rate of flow may be varied. During the flow, watch the top of the weighing tube for escaping vapors, and, as soon as any are seen, slow down the flow. Test the balance swing to make certain shifting ice in the upper part does not cause sufficient friction against the Saran tube to hinder the swing. Continue the flow of acid sample until the 40 grams is approximately balanced; then close the cylinder valve. After a delay of 15 seconds, disconnect the Saran tube and drop it carefully into the sample weighing tube, which is then stoppered tightly.

Weigh the sample tube accurately on the torsion balance, adding only analytical weights, and record the additional weight over the ice weight as the sample weight. Mix thoroughly by careful

inversion until all the ice melts, being certain to keep the tube tightly stoppered, so that none of the solution is lost before the ice melts and the solution becomes homogeneous. Remove the Saran tube and restopper without delay to prevent escape of sulfur dioxide. (The well-mixed diluted acid clinging to the Saran tube will be of no consequence, since aliquot weights will be taken for analysis.) Clean and dry the Saran tube at once. Proceed with determinations covered below without delay, using aliquot portions taken by weight.

#### ANALYTICAL DETERMINATIONS

**SULFUR DIOXIDE.** This constituent must be the first one determined, because the opening of the weighing tube for taking the other aliquot samples may result in loss of sulfur dioxide. Determination should be made as promptly as possible, avoiding standing in the sample bottle prior to analysis.

Provide a well-waxed 250-ml. beaker and a Bakelite, or Saran, stirring rod. To this beaker add 50 ml. of water and exactly 10 ml. of standard 0.1 *N* iodide-iodate solution (1). This iodine solution should be standardized at frequent intervals to ensure maximum accuracy. Weigh on a torsion balance. Place a 50-gram weight on the balance pan, then pour an aliquot portion of the sample into the beaker carefully until slightly overbalanced. Weigh accurately to  $\pm 0.5$  gram. Back-titrate the excess liberated iodine with standard 0.1 *N* thiosulfate, using starch solution as indicator. If no color appears upon adding starch, titrate with iodide-iodate to a blue color. Calculate the percentage of sulfur dioxide in the hydrofluoric acid as follows:

Weight of sample =

$$\text{weight of aliquot} \times \frac{\text{weight of anhydrous HF}}{\text{weight of ice} + \text{weight of anhydrous HF}}$$

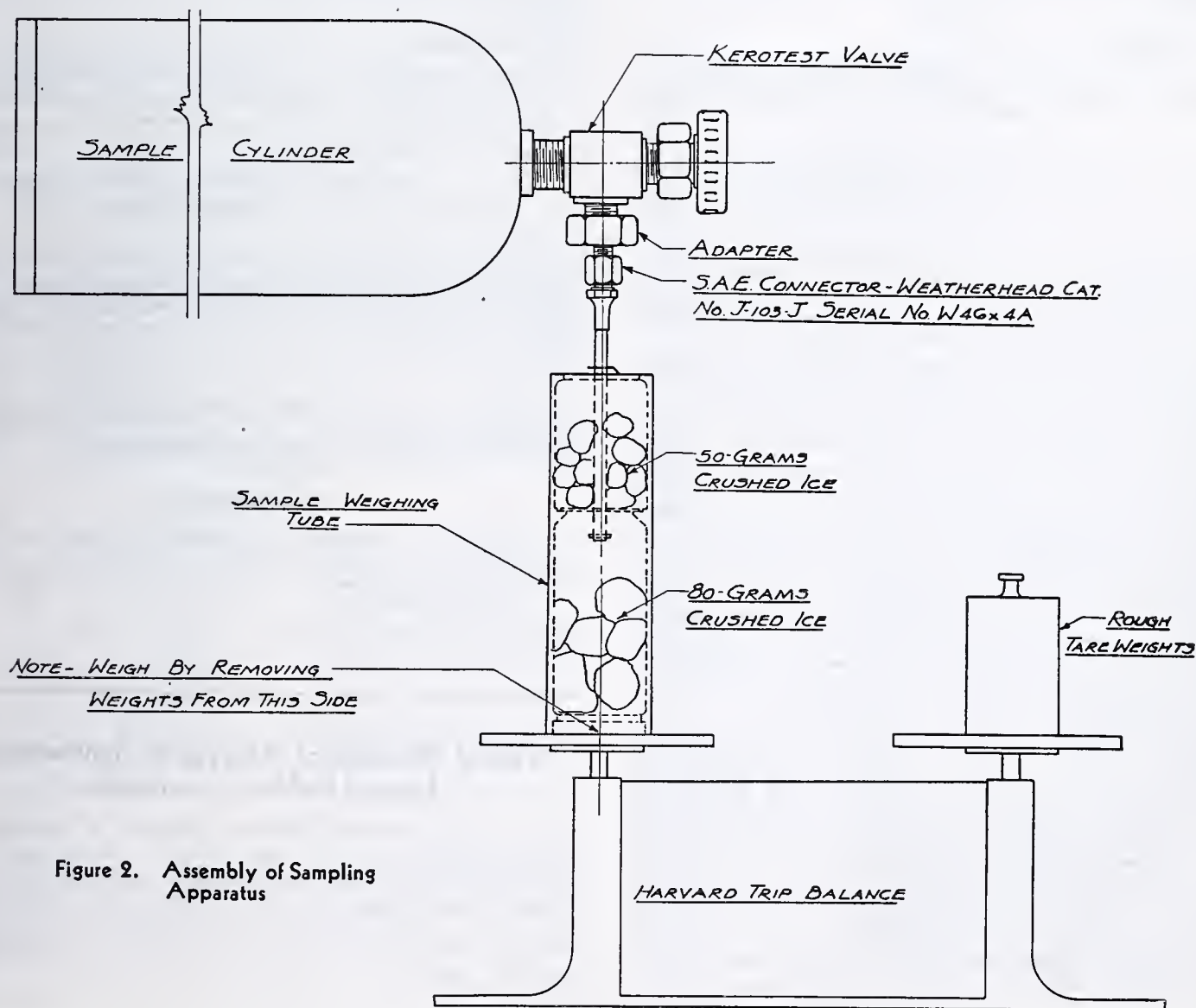


Figure 2. Assembly of Sampling Apparatus



$$\% \text{SO}_2 = \frac{\text{ml. of iodate solution} \times \text{normality} \times 3.203}{\text{weight of sample}}$$

**TOTAL ACIDITY.** Weigh a dried platinum weighing bottle, with cover, on an analytical balance. (Type bottle as shown in Figure 3 may be used.) Transfer 7 to 8 ml. (7 to 8 ml. of the diluted acid will require between 75 and 95 ml. of *N* sodium hydroxide for titration) of the diluted acid sample with a Saran dropping pipet fitted with a rubber bulb at the top. Cover the weighing bottle and reweigh.

Transfer about 25 ml. of distilled water to a 200-ml. platinum dish. (A silver or Monel dish, or a 250-ml. glass beaker coated with wax, serves as an acceptable substitute.) Add 1 ml. of phenolphthalein indicator (0.1% phenolphthalein solution in denatured alcohol) and just enough *N* sodium hydroxide solution to give a pink color. (Only a fraction of a drop of *N* sodium hydroxide normally will be needed to produce a pink color.) Allow about 75 ml. of *N* sodium hydroxide solution to run from the chamber buret into the platinum dish.

Immediately submerge the weighing bottle in the platinum dish and loosen the cover. Stir, and titrate with *N* sodium hydroxide to the first permanent pink color of the indicator. Heat the solution to boiling and if the color fades, add more sodium hydroxide to the first permanent pink color. (If a wax-lined beaker is used, the solution must be transferred to an uncoated beaker before heating.)

#### Calculations.

Weight of sample =

$$\text{weight of aliquot} \times \frac{\text{weight of anhydrous HF}}{\text{weight of ice} + \text{weight of anhydrous HF}}$$

$$\% \text{ total acidity as HF} = \frac{\text{ml. of NaOH} \times \text{normality} \times 0.02 \times 100}{\text{weight of sample}}$$

**HYDROFLUOSILICIC ACID.** Weigh 50 grams of diluted hydrofluoric acid in a platinum beaker (dull finish preferred) on a platform balance ( $\pm 0.5$ -gram accuracy). Add 0.2 gram of sodium chloride and stir until the salt has dissolved. Place the beaker on a steam bath and evaporate to dryness.

Add 25 ml. of distilled water and stir until the solids have dissolved. Add 2 grams of potassium chloride and again stir until dissolved. Add 1 ml. of phenolphthalein indicator.

Place the beaker in an ice bath and allow to cool for at least 15 minutes. Carefully titrate the cold solution with silica-free *N* sodium hydroxide until the end point has nearly been reached. (If silica-free alkali is not readily available, the silica content of the alkali used may be determined and subtracted as a blank.) Complete the titration with 0.1 *N* silica-free sodium hydroxide to the first pink color that persists for at least 15 seconds. Neglect the amount of alkali added to this point. (It is desirable to neutralize most of the acid fluorides with *N* alkali and to finish the neutralization with 0.1 *N* alkali as directed. If dilute sodium hydroxide is used for the entire neutralization, the large volume required dilutes the solution, and also raises its temperature. Both of these factors lead to a fading endpoint; some of the silica is hydrolyzed, and the results tend to be low.)

Heat the solution to boiling and titrate with 0.1 *N* sodium hydroxide to a pink end point. Reheat to boiling and finish the titration of the hot solution to the first pink which persists for 45 seconds. With less than 0.1% of silica, this second heating is not necessary. Record the volume of sodium hydroxide used for the hot titration only, which is equivalent to the silica. (Appreciable quantities of iron interfere with hydrofluosilicic acid determination, causing low values, as shown in the data table below. Present experience indicates the iron content of anhydrous hydrofluoric acid is generally less than 0.01%, as iron, on anhydrous hydrofluoric acid basis, even when the acid is shipped or stored in steel containers. However, when iron content is 0.02%, or higher, a correction should be applied based upon the amount of iron present.)

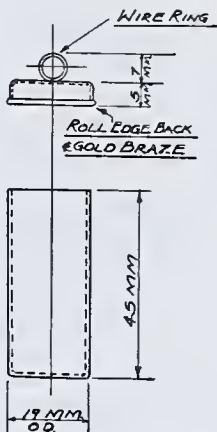


Figure 3. Platinum Weighing Bottle  
Approximate weight, 22 grams

#### Calculations.

Weight of sample =

$$\text{weight of aliquot} \times \frac{\text{weight of anhydrous HF}}{\text{weight of ice} + \text{weight of anhydrous HF}}$$

% hydrofluosilicic acid =

$$\frac{\text{ml. of 0.1 N NaOH} \times \text{normality} \times 0.036 \times 100}{\text{weight of sample}}$$

Fe Added Grams	%	H <sub>2</sub> SiF <sub>6</sub> Found Grams	%	Error Caused by Iron, % H <sub>2</sub> SiF <sub>6</sub>
0.003	0.02	0.198	0.132	0.000
0.006	0.04	0.188	0.125	0.007
0.009	0.06	0.172	0.115	0.017
0.012	0.08	0.151	0.101	0.031
0.015	0.10	0.137	0.091	0.041
		0.125	0.083	0.049

**SULFURIC ACID** (Sulfuric Acid and Fluosulfonic Acid, Calculated as Sulfuric Acid). Weigh on a torsion balance a 50.0-gram aliquot of the sample into a 75-ml. platinum dish and evaporate to apparent dryness on a water bath. Add 10 ml. of water and evaporate again to dryness on the water bath. Repeat the evaporations with water until no odor of hydrofluoric acid is detected when hot, then add water and evaporate once more. Usually two evaporations with water are sufficient for sulfuric acid contents below 0.1%.

When all the hydrofluoric acid has been expelled, add 25 ml. of carbon dioxide-free water, 1 gram of sodium fluoride, and 1 gram of potassium oxalate. Titrate with 0.1 *N* alkali using phenolphthalein indicator. The titration is equivalent to sulfuric and fluosulfonic acid. Calculate as sulfuric acid and report to two significant figures.

weight of sample =

$$\text{weight of aliquot} \times \frac{\text{weight of anhydrous HF}}{\text{weight of ice} \times \text{weight of anhydrous HF}}$$

% (H<sub>2</sub>SO<sub>4</sub> + HSO<sub>3</sub>F) as H<sub>2</sub>SO<sub>4</sub> =

$$\frac{\text{ml. of 0.1 N NaOH} \times \text{normality} \times 0.049 \times 100}{\text{weight of sample}}$$

*Note.* The sodium fluoride should be fluosilicate-free and neutral. It is added to prevent the hydrolysis of iron and aluminum salts. Potassium oxalate is added to fix salts of copper, nickel, lead, etc. Both these reagents may be omitted when the metal impurities are absent.

**DETERMINATION OF WATER.** For the alkylation grade of anhydrous hydrofluoric acid, water is regarded as the difference from 100%, subtracting the assay and impurities.

#### LITERATURE CITED

- (1) Lange, N. A., "Handbook of Chemistry", 4th ed., p. 997, Sandusky, Ohio, Handbook Publishers Co., 1938.
- (2) Manufacturing Chemists' Association, Manual Sheet H-2 (adopted 1944).
- (3) *Ibid.*, TC-5 (adopted 1943).

## Physical Methods of Analysis of Synthetic and Natural Rubber—Correction

In the article "Physical Methods of Analysis of Synthetic and Natural Rubber" [IND. ENG. CHEM., ANAL. ED., 16, 9 (1944)] under the section "Dissolving Rubber Hydrocarbon and Separating It from Compounding Ingredients", page 13, Method II, the directions should read: "Sheet the sample to a thickness of approximately 0.0375 cm. (0.015 inch) on a tight cold 15 × 30 cm. (6 × 12) inch laboratory mill."

A. R. DAVIS



# Accelerated Method for Determining Moisture Absorption

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An accelerated method for moisture-absorption measurements is described. Factors such as temperature, the size, depth, and number of dishes, and the arrangement in the humidity chamber influence the final results. Careful standardization of all conditions, therefore, is essential in order to obtain reproducible results. Considerable time can be saved by the accelerated method in making equilibrium moisture-absorption measurements of fertilizers.

THE accurate evaluation of the moisture-absorption tendencies of fertilizer materials and mixed fertilizers is of great importance in predicting the behavior of these materials in storage under humid conditions. Ordinarily, determinations are made by exposing tared samples to humidified air in a desiccator containing either a saturated salt solution or a sulfuric acid solution that gives the desired relative humidity. These tests are generally carried out under conditions existing in the laboratories, with the samples in weighing bottles or watch glasses and without agitation of the air in the desiccator. This method is referred to as the static method in the following discussion.

Results of such determinations are usually not consistent nor readily reproducible. For this reason a common fertilizer material such as sodium nitrate or ammonium sulfate, is often included in each series of determinations to serve as a standard for comparison. Lindsay (1) and others have raised objections to making moisture-absorption measurements in a stagnant atmosphere. The present paper presents a study of the factors causing these irregularities and describes an accelerated method in which readily reproducible values are obtained by means of a careful standardization of conditions. This method was developed about three years ago in this laboratory and has been in constant use.

## FACTORS AFFECTING RATE OF MOISTURE ABSORPTION

Experiments were made by the ordinary static method to determine the influence of several obvious factors, such as temperature, size of dishes, etc., on moisture absorption. In these tests, carried out under laboratory conditions, 4-gram samples of wax-treated ammonium nitrate in 6.3-cm. (2.5-inch) watch glasses, unless otherwise stated, were placed on the porcelain plate with 0.6-cm. (0.25-inch) perforations in a 30-cm. (12-inch) desiccator

containing a saturated solution of sodium nitrate which gives a relative humidity of 74.4% at 25° C. During these tests the temperature in the laboratory fluctuated between 20° and 25° C. The results obtained are tabulated in Table I.

The effect of the number and depth of dishes on moisture absorption is shown in Tables II and III. The data reveal that the temperature, the size, depth, and number of dishes, as well as the arrangement in the humidity chamber, all influence the final results. Careful standardization of all conditions is, therefore, necessary if reproducible results are to be obtained, when determining rates of moisture absorption.

Table II. Effect of Number of Samples in Humidity Chamber

No. of Samples in Humidity Chamber at One Time <sup>a</sup>	Average % Moisture Absorbed	
	92.9% R.H.	72.4% R.H.
1	15.68	5.22
2	13.25	3.86
3	12.08	3.36
4	10.75	3.23
5	9.62	2.58

<sup>a</sup> 4-gram samples of  $\text{NH}_4\text{NO}_3$  in 2-inch dishes exposed for 6 hours at 30° C.

Table III. Effect of Depth of Sample Dishes

Depth of Sample Dish <sup>a</sup> Inch	Moisture Absorbed	
	92.9% R.H. %	72.4% R.H. %
0.25	42.06	13.91
0.5	30.02	9.04
0.75	25.00	7.53
1	19.84	5.96

<sup>a</sup> Four 4-gram samples of  $\text{NH}_4\text{NO}_3$  in 2-inch dishes of various depths in same humidity chamber for 24 hours at 30° C.

## ACCELERATED METHOD

APPARATUS. The humidity chamber (shown in Figure 1) used in the accelerated method consists of a metal can, A [an inverted lower portion of a straight-walled, 23-kg. (50-pound) lard can, 20.5 cm. (7 inches) high and 30 cm. (12 inches) in diameter], with an aluminum top, B, having six equally spaced holes (6.25 cm., 2.5 inches, in diameter), through which the samples are introduced into the chamber.

The small induction motor, I is mounted on a Bakelite plate, J, insulating the humidity chamber against the heat developed in the motor. Shaft K of the four-blade aluminum fan (7 inches in diameter) is similarly insulated by means of a Bakelite coupling, M. Zink (2) used small fans on pivots inside a desiccator and induced them to rotate by means of permanent magnets passing near the outside of the desiccator.

The joint between the aluminum top and the can is made airtight by means of two rubber gaskets, T, one cemented to the aluminum top and the other to the top edge of the can.

The motor is regulated to run at approximately 350 r.p.m. by means of a voltage regulator. A much higher fan speed than this is not recommended because it will cause the temperature in the humidity chamber to rise unduly, as the result of air resistance against the action of the fan. The use of a metal chamber, raised above the table by legs N, permits rapid dissipation of excess heat from this source. In a constant-temperature room where the air is well circulated to maintain uniform temperature, the rise in temperature in the humidity chamber amounts to about 0.2° C. For accurate work, this can be compensated by lowering the room temperature correspondingly, in order to keep the chamber at exactly 30° C. or other desired temperatures.

Dish G (20-cm., 8-inch, Pyrex cake plate) contains the saturated salt solution, H, with an excess of the solid salt. The aluminum baskets, D, holding the sample dishes (with the covers removed) are suspended from glass hook E, held by rubber stoppers F, arranged as shown in Position I. Position II is drawn to show the details of the aluminum basket and hook arrangement only.

The shallow flat-bottomed sample dishes, having an inside area of 17.16 sq. cm. (2.66 sq. inches), are made by sealing Pyrex rings,

Table I. Moisture Absorption by Static Method under Laboratory Conditions

Series No.	Moisture Absorbed in 24 Hours	Remarks <sup>a</sup>
	%	
A-1	5.35	Desiccator containing samples placed on laboratory bench in middle of room
A-2	4.97	
A-3	5.48	
A-4	5.84	
B-1	7.80	Same as series A, except desiccator placed near window with B-1 and B-2 near and B-3 and B-4 away from window
B-2	7.10	
B-3	5.28	
B-4	5.63	
C-1	5.68	Same as series A, except desiccator protected from drafts to obtain more uniform temperature in desiccator
C-2	5.67	
C-3	5.40	
C-4	5.72	
D-1	4.86	Same as series C, except D-1 and D-2 in 2.5-inch dishes, D-3 and D-4 in 3-inch dishes
D-2	5.26	
D-3	6.59	
D-4	6.82	
E-1	11.51	Same as series C, except perforated plate in desiccator removed and samples suspended from Bake- lite top
E-2	12.83	
E-3	12.57	
E-4	10.48	

<sup>a</sup> Four 4-gram samples of wax-treated  $\text{NH}_4\text{NO}_3$  in 2.5-inch watch glasses in desiccator at one time.



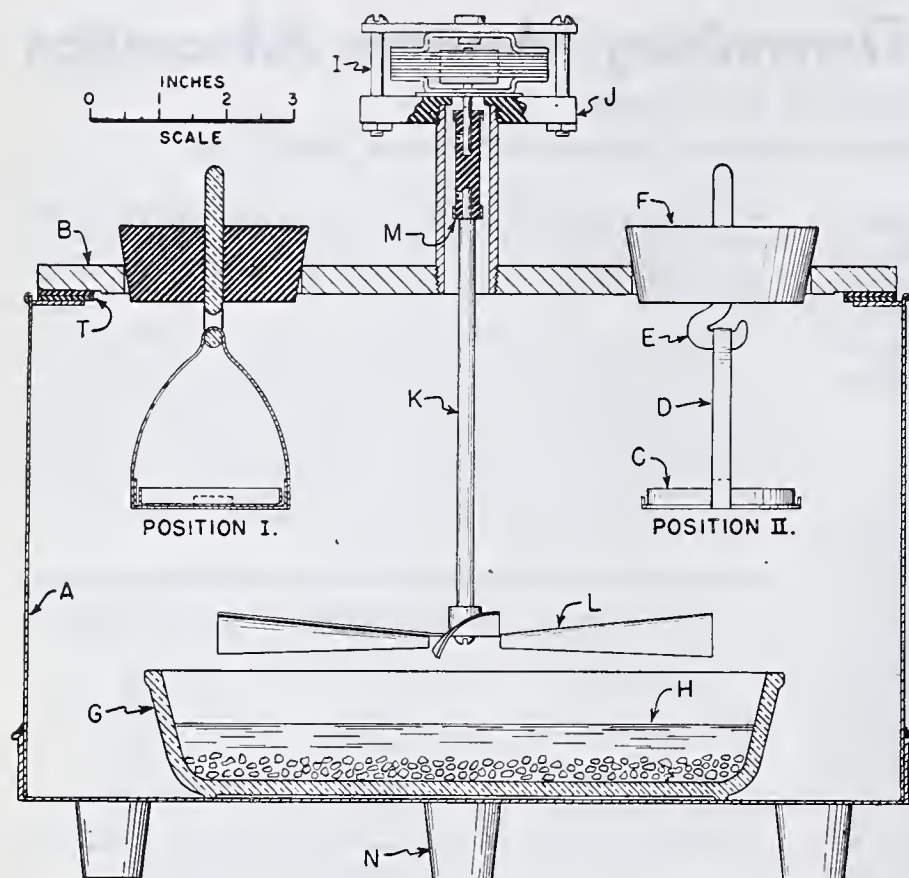


Figure 1. Humidity Chamber

Table IV. Moisture Absorption Rate by Accelerated Method at 30° C.

No.	Sample <sup>a</sup>	Relative Humidity in Chamber %	Moisture Gained in Various Time Intervals					
			1 hour	2 hours	3 hours	4 hours	5 hours	6 hours
1	NaNO <sub>3</sub>	75.2	0.86	1.73	2.71	3.60	4.39	5.49
			0.87	1.75	2.71	3.77	4.46	5.57
2	NaNO <sub>3</sub>	75.2	0.82	1.75	2.72	3.63	4.41	5.42
			0.82	1.77	2.70	3.61	4.45	5.36
3	CO(NH <sub>2</sub> ) <sub>2</sub>	75.2	1.72	3.14	4.36	5.61	6.69	7.66
			1.78	3.16	4.39	5.70	6.71	7.80
4	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	84	1.30	2.71	3.99	5.41	6.82	8.35
			1.26	2.60	3.94	5.31	6.87	8.38
5	NH <sub>4</sub> NO <sub>3</sub> , sample A	65.2	..	6.82	..	13.23	..	19.21
			..	6.77	..	13.40	..	19.52
6	NH <sub>4</sub> NO <sub>3</sub> , sample B, wax-coated	72.4	..	0.36	..	0.63	..	0.87
			..	0.39	..	0.67	..	0.92
7	NH <sub>4</sub> NO <sub>3</sub> , sample B, wax-coated	81.4	..	0.64	..	1.18	..	1.87
			..	0.71	..	1.20	..	1.62
8	NH <sub>4</sub> NO <sub>3</sub> , sample B, wax-coated	96.3	..	1.41	..	2.30	..	3.09
			..	1.34	..	2.15	..	2.90
9	Fertilizer X	72.4	0.43	0.39	0.88	1.22	3.56 <sup>b</sup>	3.72 <sup>c</sup>
			0.61	0.34	0.87	1.22	3.58	3.73
10	Fertilizer Y	65.2	1.98	2.91	3.56	3.93	..	..
			1.70	2.48	2.98	3.52	..	..

<sup>a</sup> 4-gram samples in 2-inch dishes used in all tests, except No. 2, 2.5-gram samples.

<sup>b</sup> 21-hour result.

<sup>c</sup> 23-hour result.

0.6 cm. (0.25 inch) high, onto flat Pyrex plates, 5 cm. (2 inches) in diameter. The covers for these dishes are inverted watch glasses of the same size, with glass knobs cemented to them to facilitate handling. Since such Pyrex plates are not easily obtained now, aluminum dishes (as shown in Figure 2) of similar dimensions are also used. For protection against the corrosive action of certain types of fertilizers, the inside surfaces of these aluminum dishes are well lacquered.

Figure 3 shows the details of the aluminum spreading device used to distribute the samples uniformly on the dishes. It consists, essentially, of a disk, *O*, with two rows of pins, 0.3 cm. (0.125 inch) in length at staggered distances from the center of the disk and intersecting at right angles to each other as shown in section *A'-A'*, Figure 3.

Figure 4 shows the arrangement of the leveling device on top of the sample in a dish. This device consists of a flat aluminum disk, *Q*, slightly smaller in diameter than that of the dish, with a platform, *R*, of similar size to take a kilogram weight, *S*.

#### PROCEDURE

**DETERMINATION OF RATE OF MOISTURE ABSORPTION.** The tared dish (with cover removed) containing the sample is placed under the spreading device, as shown in Figure 3. Disk *O* is then rotated 7 or 8 revolutions at a moderate speed, while the sample dish is being turned in the opposite direction. This procedure is repeated with a change in the direction of rotation. If the spreading disk is properly adjusted, the pins should just touch the surface of the leveled sample in the dish. If furrows are formed in the sample, the spreading disk is too low and should be raised.

Next the leveling device with a kilogram weight is placed on top of the spread sample, as shown in Figure 4, and allowed to stand for 2 minutes (arbitrarily chosen), so as to produce a uniformly smooth surface and packing of the sample in the dish. This leveling step is necessary for very accurate determinations because the rate of moisture absorption depends on the surface area exposed to the humid atmosphere.

If the sample is in coarsely granular form, it is impossible to obtain a uniformly smooth surface as with finer materials. Such coarse granules can be spread simply with a spatula.

With the fan going at the proper speed, the samples are placed in the humidity chamber, as shown in Position I in Figure 1. At the end of a desired time interval (1 or 2 hours) the samples are taken out and weighed with the covers on to determine the increases in weight and then replaced in the chamber for another interval before reweighing. The increases in weight for the various time intervals are calculated as the moisture-absorption rate in per cent of the sample.

**EQUILIBRIUM MOISTURE-ABSORPTION MEASUREMENTS.** The same procedure is followed, except that it is sufficient to spread the samples uniformly with the aid of a spatula, and to keep them in the humidity chamber for 2 days. This is sufficient time for equilibrium to be established in practically all cases. Fertilizers containing an unusually large amount of hygroscopic components, however, may require an extra day. Such fertilizers can be easily recognized by their high absorption values.

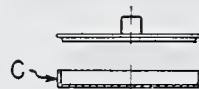


Figure 2. Sample Dish with Cover

Table V. Comparison of Results Obtained by Static and Accelerated Methods

No.	Sample <sup>a</sup>	% Moisture Absorbed			
		Static Method <sup>b</sup> 24 hours	Accelerated Method 2 hours	4 hours	6 hours
1	NH <sub>4</sub> NO <sub>3</sub> , tech.	20.32	10.91	20.20	29.22
2	NH <sub>4</sub> NO <sub>3</sub> wax-coated and dusted, sample A	4.96	2.97	4.35	5.46
3	NH <sub>4</sub> NO <sub>3</sub> coated and dusted, sample B	10.61	5.74	10.74	16.08
4	NH <sub>4</sub> NO <sub>3</sub> wax-coated	0.76	0.48	0.71	0.92
5	NH <sub>4</sub> NO <sub>3</sub> dusted only	19.16	10.29	19.82	29.15

<sup>a</sup> 4-gram samples in 2-inch dishes exposed to atmosphere of 72.4% relative humidity at 30° C.

<sup>b</sup> Fan in humidity chamber stopped.

Table VI. Effect of Temperature on Moisture Absorption

Sample <sup>a</sup>	Temperature ° C.	Moisture Absorbed %
Urea <sup>b</sup>	29.5	2.86
	30.0	3.16
	30.5	3.43
NaNO <sub>3</sub> <sup>c</sup>	29.5	1.76
	30.0	1.90
	30.5	2.07

<sup>a</sup> Samples in 2-inch dishes exposed to atmosphere of 75% R.H. for hours.

<sup>b</sup> 2.5-gram samples.

<sup>c</sup> 3.7-gram samples.



**Table VII. Relation between Moisture Absorption and Fineness of Sample**

Fineness of Sample <sup>a</sup> Mesh	Moisture Absorbed %
20-48	1.78
48-100	1.78
100-150	1.77
150-200	1.79
Through 200	1.77

<sup>a</sup> 2.5-gram samples of NaNO<sub>3</sub> in 2-inch dishes exposed to atmosphere of 75% R.H. at 30° C. for 2 hours.

**Table VIII. Relation between Moisture Absorption and Amount of Sample Used**

No.	Sample Used, A <sup>a</sup> Grams	Moisture Absorbed, B Gram	Moisture Absorbed, B/A %
1	5.0	0.0462	0.93
2	4.5	0.0456	1.02
3	4.0	0.0460	1.15
4	3.5	0.0453	1.30
5	3.0	0.0458	1.53
6	2.5	0.0463	1.85
7	2.0	0.0453	2.27
8	1.5 <sup>b</sup>	0.0387	2.58

<sup>a</sup> NaNO<sub>3</sub> (48- to 100-mesh) in 2-inch dishes exposed to atmosphere of 75% R.H. for 2 hours at 30° C.

<sup>b</sup> Just enough sample to cover dish at beginning of test.

Because of variations in the density of the various materials and the need for an approximately constant volume of sample, the amount of fertilizer taken for such a test has to be adjusted in some extreme cases, but in general 4-gram samples work out well for the size of dish chosen. The relative humidity used likewise has to be chosen according to requirements in individual cases. For testing ammonium nitrate that has undergone various conditioning treatments, 72% relative humidity has been adopted. This represents about the average condition encountered locally.

### EXPERIMENTAL RESULTS

**RATE OF MOISTURE ABSORPTION.** In order to test the accuracy and reproducibility of the results obtained by the accelerated method, the moisture-absorption rates of a number of fertilizer materials and mixed fertilizers were determined in duplicates at different relative humidities at 30° C. The results, tabulated in Table IV, show good agreements between duplicate samples. Experiments 1 and 2, made at different times and in different humidity chambers, show the reproducibility of such results.

Table V shows that under identical conditions as much moisture is absorbed in 4 hours by the accelerated method as in 24 hours by the static method.

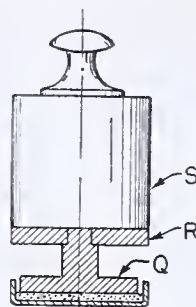
Variations of only 0.50° C. in temperature produce distinct changes in the results, as shown by Table VI. These are, un-

doubtedly, brought about by change in the relative humidity over the salt solution as well as in the absorption rate of the samples.

Sodium nitrate samples of different particle sizes, ranging from 20- to 48-mesh to through 200-mesh, give identical absorption values when tested under the same conditions (Table VII). The reason for this is that the absorption values obtained are actually not values for the solid phase, but for the saturated solution surrounding the solid particles. Therefore, once a thin layer of

saturated solution is formed over the leveled surface layer of solid particles, regardless of the size of the particles, the rate of absorption of the saturated solution surrounding the solid is the same, as long as there is enough solid phase present to keep this solution saturated; otherwise the absorption rate will gradually decrease as the solution becomes more dilute.

For the same reason the amounts of moisture absorbed by varying sized samples (1.5 to 5 grams) of 48- to 100-mesh sodium nitrate are practically identical under the same conditions, although the percentage of moisture absorbed (column 4 Table VIII) varied from 0.93 to 2.58%. It appears that the amount absorbed is governed by the surface exposed rather than by the amount of sample. The low value of experiment 8 was due to insufficient amounts of solid salt present to keep the solution satu-

**Figure 4. Sample Dish with Leveling Device****Table IX. Constancy of Moisture Absorption per Unit Weight of Sample per Unit Area of Surface Exposed**

Sample Dish	Area in Dish Sq. in.	Sample in Dish <sup>a</sup> Grams	Gain per 2-Hour Intervals <sup>b</sup> 1st %	2nd %	3rd %
A	1.5920	3.5942	0.95	0.82	0.83
B	2.6577	6.0	0.84	0.82	0.81

<sup>a</sup> Same weight of sample per sq. inch in each dish.

<sup>b</sup> At 75% relative humidity and 30° C.

**Table X. Total Moisture in Fertilizers at Equilibrium**

Fertilizer Sample <sup>a</sup>	% Moisture in Original Samples	59.4% R.H.	65.2% R.H.	72.5% R.H.
A	3.86	2.94 3.13	5.76 5.70	19.45 19.26
B	4.61	3.42 3.61	7.47 7.50	19.86 19.79
C	4.28	3.80 3.65	9.15 9.25	20.67 20.74
D	5.76	3.35 3.55	9.72 9.82	17.93 17.97
E	5.76	4.60 4.59	12.84 12.79	20.55 20.97
F	4.08	3.08 3.05	25.37 24.85	40.87 42.78

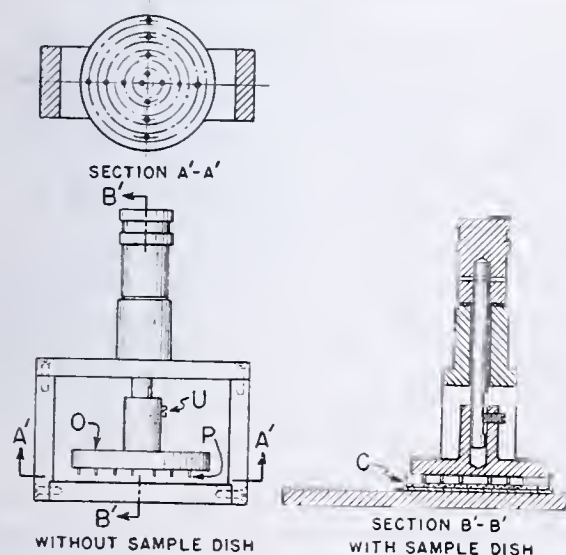
<sup>a</sup> 4-gram samples in 2-inch dishes in humidity chamber for 48 hours at 30° C. at three relative humidities.

<sup>b</sup> % total moisture at equilibrium = % moisture absorbed + % moisture in original sample.

**Table XI. Comparison of Time Required to Come to Equilibrium at 30° C.**

Fertilizer Sample <sup>a</sup>	Relative Humidity in Chamber	Method Used	2 days	% Moisture Absorbed 4 days	6 days	8 days	11 days
A	59.4	Accelerated	4.68				
A	59.4	Static	3.63	4.24	4.51	4.66	4.62
A	65.2	Accelerated	17.00				
A	65.2	Static	12.97	15.50	16.65	17.05	17.13
A	72.4	Accelerated	29.42				
A	72.4	Static	19.55	24.57	27.20	28.67	29.49
B	59.4	Accelerated	2.65				
B	59.4	Static	1.61	2.00	2.14	2.33	2.48

<sup>a</sup> 4-gram samples in 2-inch dishes used.

**Figure 3. Spreading Device**



rated during the test. In similar tests with coarse granular materials having large interstices between granules, so that the moist air can penetrate deeply into the mass, different absorption results may be obtained with different amounts of sample taken, because in such cases the effective area of the samples exposed to the moist atmosphere also varies.

Various sizes of dishes can be used to give identical results, as demonstrated by Table IX, provided the same unit weight of sample is taken per unit area of surface exposed to the humid atmosphere. This applies, however, only to dishes having flat bottoms and straight side walls. It does not hold in the case of watch glasses, because in this case the solution around the edges may become locally undersaturated while there is still solid salt in the center of the dish.

**EQUILIBRIUM MOISTURE-ABSORPTION MEASUREMENTS.** Table X shows that the method gave good precision on the total moisture content of several fertilizers in equilibrium with three relative humidities at 30° C. The particular relative humidities were chosen because most of the common fertilizers begin to absorb moisture around 65% relative humidity.

Results, shown in Table XI, reveal that considerable time can be saved by the accelerated method in making equilibrium moisture-absorption measurements of fertilizers.

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- (2) Zink, F. J., *IND. ENG. CHEM., ANAL. ED.*, 7, 442 (1935).

## Determination of Spoilage in Protein Foodstuffs, with Particular Reference to Fish

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Based on the determination by an aeration technique of volatile reducing substances, a method has been developed for estimation of spoilage in proteinaceous foodstuffs, with particular reference to fish. The oxidizing solution is a 0.1 *N* potassium permanganate solution in *N* sodium hydroxide. Values for California sardines, mackerel, and tuna at various stages of spoilage are given. Further applications of the procedure for the estimation of volatile principles in other foods and food products are indicated.

**W**IDELY varying methods have been proposed and employed for the evaluation of "borderline" freshness or incipient spoilage in fish and fish products where subjective organoleptic opinions are likely to differ and definite decisions are made with difficulty.

The fact that new methods and modifications of old ones are reported from time to time implies the lack of a sufficiently sensitive and reliable procedure for the determination of the state of incipient spoilage.

The majority of chemical methods used for this purpose are dependent upon the presence of specific substances, or groups of related substances, such as indole, hydrogen sulfide, volatile nitrogenous bases, and volatile acids. The production of these compounds, predominantly by bacterial action, is dependent upon the particular flora present. Furthermore, considering the wide range of bacterial species with diverse biochemical activities which are encountered in and around fish during spoilage, the mere absence of specific products will not necessarily indicate the actual state of freshness.

The use of a method designed for the detection of a specific type of spoilage product assumes that it has been produced in greater amounts than all others. This may or may not be the case, since fish may be spoiled and still not yield a positive test for the substance upon the presence of which a particular method depends, because the organisms responsible for the decomposed condition have not produced the compound.

Fresh fish have relatively little odor, and as fish undergo spoilage unpleasant odoriferous substances are formed, the amount and variety being dependent upon the extent and kind of decomposition.

Investigators responsible for evaluation of the condition of fish and other food products, recognizing these facts, have resorted to the more simple, rapid, and relatively sensitive organoleptic

method. However, certain factors adversely affect its use, such as the influence of temperature on odor, the masking of odors, personal opinion or preference, the ability of one person to detect an odor more readily than another, and olfactory fatigue.

A chemical method to serve as a sensitive and reliable measure of the freshness of fish has been developed, in which the volatile substances largely responsible for odor are estimated and by which the state of preservation of a fish sample can be determined. A close correlation has been observed between the chemical values and the organoleptic judgment—namely, low values for fresh material and progressively higher values for material after progressive stages of spoilage. However, changes in fish have been determined chemically before they could be detected organoleptically.

This report describes the principle and operation of the method, with data illustrative of its application to various proteinaceous foodstuffs. A more detailed account of its application to determination of spoilage in various Pacific Coast and other fishes, as well as an extensive critical review of the literature on the spoilage of fish and fish products, will be presented in subsequent papers.

#### PRINCIPLE OF THE METHOD

The method is based on the fact that during spoilage volatile odoriferous substances are produced in fish. The act of smelling constitutes essentially the passage of volatile odorous materials admixed with air from the sample to the sensory nerves of the nose. It seems reasonable to expect that these volatile constituents can be carried off from as ample by a current of air into suitable reagent and there determined.

Previous studies related to aeration of volatile substances have included the determination of hydrogen sulfide and ammoniacal bases. These and other volatile compounds, such as indole and fatty acids, have more often been separated by distillation procedures (steam and vacuum). However, as far as the authors are aware, there are no other published studies on the use of aeration for determining the volatile constituents of foodstuffs as a whole in relation to the spoilage problem.

The use of an oxidizing agent for estimation of the volatile substances was adopted in view of the fact that most types of volatile organic compounds which conceivably might be formed during spoilage are oxidizable. The reducing action of organic com-



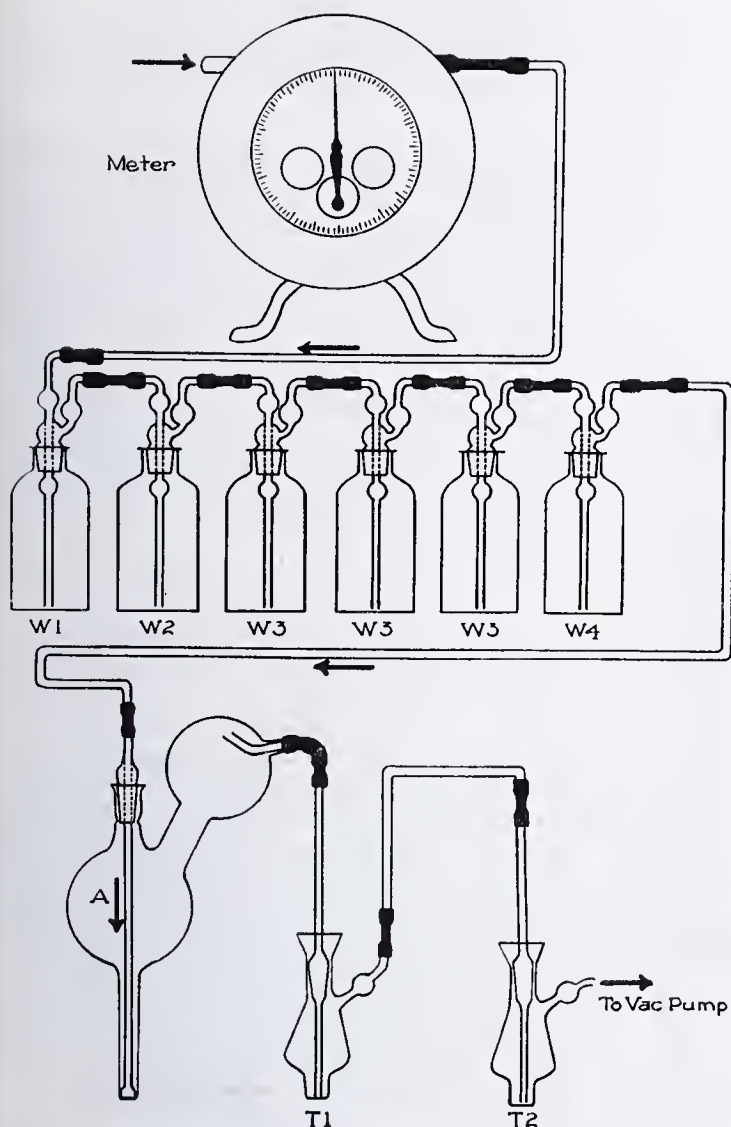


Figure 1. Diagram of Original Apparatus

- A. Aeration flask  
 T1, T2. Reaction vessels  
 W1. Concentrated  $\text{H}_2\text{SO}_4$   
 W2, W4. Redistilled water  
 W3. 10% alkaline  $\text{KMnO}_4$  or activated carbon  
 Meter. Wet-test air meter

pounds has been utilized frequently in their determination. The work of Quitmann (1) on the determination of the reducing value of air as a measure of its cleanliness, and of Stamm (2) on the estimation of organic compounds, may be cited in this connection.

#### EXPERIMENTAL

**APPARATUS.** The apparatus assembled for use is shown in Figures 1 and 2. Figure 1 illustrates the apparatus as originally set up, using a sensitive gas meter to measure the air volume aspirated through the sample. A more compact and portable two-unit sample is shown in Figure 2, using Pyrex flowmeters, which were calibrated against the air meter, to measure the air volume. Figure 3 shows the aeration flask, A, for the sample, its air-inlet tube, and two reaction vessels, T1 and T2, for the alkaline potassium permanganate oxidizing solution with their air-inlet tubes.

A consists of two bulbs of 1-liter and 500-ml. capacities and a  $20 \times 125$  mm. Pyrex tube sealed onto the 1-liter bulb. The upper 500-ml. bulb is connected to the lower 1-liter bulb by a 60-mm. neck and has an outlet tube connected to an inner spray and foam trapping tube. The two bulbs are necessary to take care of excessive frothing which sometimes occurs, as, for example, with very fresh fish samples. The air-inlet tube of the aeration flask is kept in place with an interchangeable 24/40 standard-taper ground-glass male joint and its end is closed with a sintered-glass plate slightly smaller than the diameter of the tube portion.

The reaction vessel, T, is a Pyrex 125-ml. Erlenmeyer with a deep gutter around the neck (an iodine number determination flask), to the base of which has been sealed a  $20 \times 35$  mm.

Pyrex well. The reaction flask is fitted with an air-inlet tube which is an interchangeable ground-glass 24/40 standard-taper male joint with a reduced tube of 12-mm. diameter at both ends, one end of which is closed with a sintered-glass plate.

All rubber connections used in the apparatus were originally boiled in 5% sodium hydroxide solution and thoroughly washed with several changes of boiling redistilled water. They should be periodically rewashed.

**SAMPLING PROCEDURES.** In sampling raw fish a sufficient portion of the material under examination, such as 12 California sardines (pilchards) or 12 mackerel or a 5- to 10-cm. (2- to 4-inch) transverse cross-sectional slice from a tuna, is ground and well mixed. Portions of this ground mixture are wrapped in a double layer of cheesecloth or gauze, placed in the cylinder (C, Figure 4) of the squeezing apparatus on top of a perforated steel disk, a Chromel wire screen, a 0.3-cm. (0.125-inch) white felt pad, and a second Chromel wire screen, in the order named, and a pressure of 3 to 5 tons is applied to piston (P, Figure 4). The expressed juice is collected through the spout, S, at the base of the cylinder. The dimensions of the various parts of the squeezing apparatus are: cylinder C,  $10 \times 11.5$  cm. ( $4 \times 4.5$  inches) outer dimensions, with a 1.25-cm. (0.5-inch) wall, piston P  $7.5 \times 10.2$  cm. ( $3 \times 4$  inches) perforated disk  $7.3 \times 0.6$  cm. ( $2^{15}/16 \times 1/4$  inches). All are made of steel.

For canned fish, the entire contents of a can is squeezed by hand or in a small press of the fruit and vegetable juice extractor type, through two layers of cheesecloth or gauze, and the press liquor collected. All press juices are allowed to stand or are centrifuged to separate the aqueous solution. A uniform representative sample of the water-soluble constituents of the fish is thus obtained.

**AERATION OF SAMPLE.** Five milliliters of the fish press juice sample are pipetted into the test tube portion of flask A and aerated 40 minutes with 0.0566 cubic meter (2 cubic feet) of air, measured by the air meter, M, or by the flowmeter, F, into two reaction vessels, T1 and T2. Each reaction vessel contains 10 ml. of 0.1 N potassium permanganate solution in N sodium hydroxide solution. The air is cleaned by passage through six Drechsel gas-washing bottles containing, in order, concentrated sulfuric acid, W1, redistilled water, W2, 10% potassium permanganate in 10% sodium hydroxide solution W3 (3 bottles), and redistilled water, W4. Recently activated granular carbon has been substituted successfully for the 10% alkaline potassium permanganate solution. The air is aspirated by means of a laboratory vacuum pump through a stabilizing storage tank to eliminate stroke fluctuations. A water suction pump may be used as long as the water pressure is constant and not subject to fluctuations.

The concentrated sulfuric acid in the Drechsel bottle, during prolonged aeration, absorbs moisture and some organic matter from the air, becoming diluted and brown. When in constant use, it should be renewed every week, either by using fresh acid or by boiling down the diluted acid with a trace of an oxidizing agent, such as sodium nitrate. The 10% alkaline potassium permanganate solution eventually develops a brown precipitate and likewise should be replaced every week or two. The use of activated carbon obviates this cleaning procedure.

**OXIDIZING REAGENT.** The potassium dichromate-sulfuric acid reagent of Quitmann (1) was originally used as the oxidizing solution in the reaction vessels, T. However, it was found that a 0.1 N potassium permanganate in N sodium hydroxide was a much more sensitive reagent. An acid potassium permanganate solution was less effective than the acid dichromate mixture, while ceric sulfate in sulfuric acid was completely ineffective as an oxidizing solution at room temperature for the volatile reducing substances from fish press juice. The greater oxidizing power for organic compounds of alkaline over acid potassium permanganate solution has been reported (3). The normality of the alkali in the potassium permanganate solution affects the amount of reduction obtained; somewhat lower values were found with 0.1 N than N sodium hydroxide.

The alkaline potassium permanganate solution is prepared with water redistilled from an alkaline potassium permanganate solution in an all-glass distilling apparatus. The solution is boiled for 5 to 10 minutes, allowed to stand covered overnight at room temperature, and filtered through asbestos on a sintered-glass funnel. The alkaline potassium permanganate solution is usually made up in 2-liter quantities and stored in an amber glass bottle with a screw cap. Under these conditions, the solution is stable as long as it lasts.

**DETERMINATION OF AMOUNT OF REDUCTION.** The amount of reduction of the permanganate is determined as follows:

To 10 ml. of oxidizing reagent in vessel T, after aeration, are added 5 ml. of 6 N sulfuric acid followed by 10 ml. of 0.11 N fer-



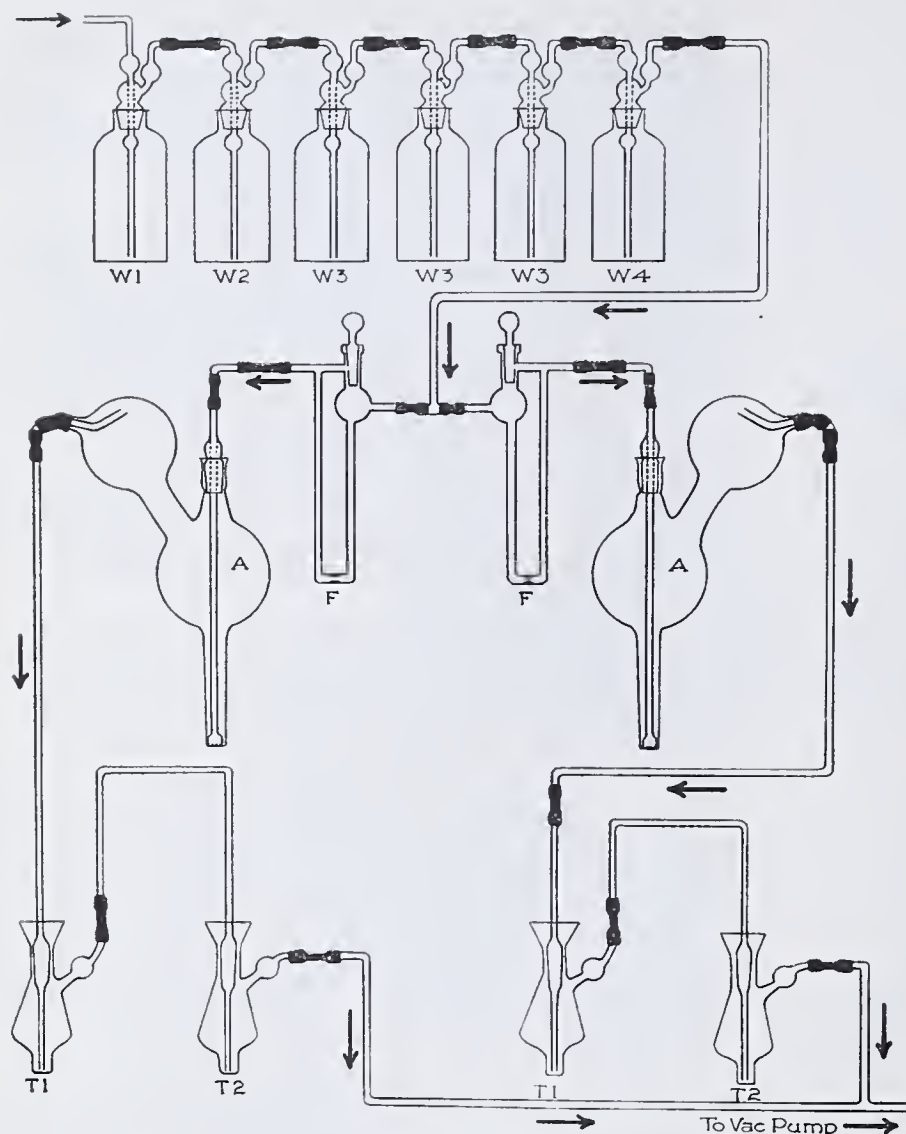


Figure 2. Diagram of Portable Two-Sample Unit

- A. Aeration flask
- F. Flowmeter
- T1, T2. Reaction vessels
- W1. Concentrated  $\text{H}_2\text{SO}_4$
- W2, W4. Redistilled water
- W3. 10% alkaline  $\text{KMnO}_4$  or activated carbon

rous ammonium sulfate in 0.01 *N* sulfuric acid. The excess of Mohr's salt is titrated with 0.01 *N* ceric sulfate in *N* sulfuric acid solution, using *o*-phenanthroline ferrous complex as indicator. Ten milliliters of the original oxidizing solution in a trap with a sintered-glass tube, after standing at room temperature for the duration of an aeration (40 minutes), are acidified with 5 ml. of 6 *N* sulfuric acid and mixed with 10 ml. of the ferrous ammonium sulfate solution, and the excess Mohr's salt is titrated with the ceric sulfate solution. This titer serves as the control for the unreacted oxidizing solution.

The difference between the test and the control titers is directly proportional to the amount of reduction of the potassium permanganate, which is expressed as microequivalents of reduction. A titer difference of 1 ml. of the 0.01 *N* ceric sulfate solution corresponds to a reduction of 10 microequivalents. The concentration of the volatile substances in a sample is directly proportional to the amount of reduction of the oxidizing agent. Studies are in progress on the determination of the reduction spectrophotometrically and preliminary experiments indicate its feasibility.

**STABILITY OF THE MOHR'S SALT SOLUTION.** The 0.11 *N* ferrous ammonium sulfate in 0.1 *N* sulfuric acid decreases in the concentration of ferrous ion on standing exposed to the air in the storage bottle. It was found that by replacing the air in and above the solution with illuminating gas, the rate of deterioration of the solution was reduced. (The authors wish to thank Paul L. Kirk, Division of Biochemistry, University of California, for this suggestion.) The stability of the solution was further markedly increased by the addition of 0.001% of sodium hydro-sulfite or sodium sulfite. To facilitate having an atmosphere of illuminating gas in contact with the solution of Mohr's salt at all times, a dispensing arrangement similar to that of a water wash-bottle was inserted into the neck of the amber glass storage

bottle, whereby the pressure of the gas is used to deliver the solution. The concentration, under these storage conditions, remains fairly constant for the duration of the 2-liter batch of solution.

**FACTORS AFFECTING RATE OF REMOVAL OF VOLATILE REDUCING SUBSTANCES (V.R.S.).**  
*Volume of Air Aspirated through Sample.* The amount of volatile reducing substances carried over into the oxidizing reagent from a sample depends, among other factors, upon the volume of air aspirated through it. Some experiments were carried out to ascertain the volume of air necessary to ensure maximum yields of volatile reducing substances consistent with efficiency of operation. As examples of reducing substances with different volatilities, aqueous solutions of varying concentrations of ethanol and of acetone were used.

The per cent removal of a volatile substance by a definite volume of air is practically constant and is independent of the concentration of the volatile substances. The magnitude of the per cent removal at any air volume is dependent upon the volatility of the substance. For example, with 0.00566 cubic meter (0.2 cubic foot) 48% of acetone, but only 11% of ethanol, is removed from various solutions. The effect of low volatility is lessened with greater volumes of air: with 2.0 cubic feet of air, 95% of acetone is removed from a solution and 86% of ethanol is carried over. This establishes a valid basis for the adoption of an arbitrary air volume which would allow efficient and practical operation of the method, without having to carry on the aeration until all the volatile material has been removed. The following data are illustrative:

With 2.0 cubic feet of air, 86 and 87% of the total volatile reducing substances were recovered, respectively, from two samples of raw sardine (pilchard) and raw tuna press juices. The actual amounts of reduction for these two samples were 129.5 and 20.6 microequivalents per 5 ml. The per cent recoveries can be regarded as uniform, considering the diversity in the

concentration of volatile reducing substances in these two preparations.

**Temperature.** A factor of possible influence is temperature, both of the sample and of the oxidizing reagent. No significant or practical advantage was found in warming the sample to 60° C. At higher temperatures, the raw press juice coagulates and tends to block the sintered-glass plate. The alkaline potassium permanganate oxidizing solution apparently undergoes some decomposition at higher temperatures, since controls with the oxidizing solution maintained (for 40 minutes) at 60° to 100° C. gave much higher values than controls kept at room temperature. The effect of temperature was not studied further.

**OTHER GASES AS ASPIRATING MEDIA.** Since gases might behave differently as sweeping agents for volatile reducing substances from aqueous solution, various gases were compared with air. No significant difference in the recovery of volatile reducing substances was found with nitrous oxide, oxygen, and nitrogen, all of which apparently act as mechanical sweeping agents for the volatile reducing substances.

**PRESSURE OF AIR DURING ASPIRATION.** In the arrangement of the apparatus described above, the air meter is at the start of the air-washing train—that is, the air is metered at atmospheric pressure and passes through the meter before entering the washing system. When the meter was placed between the second re-



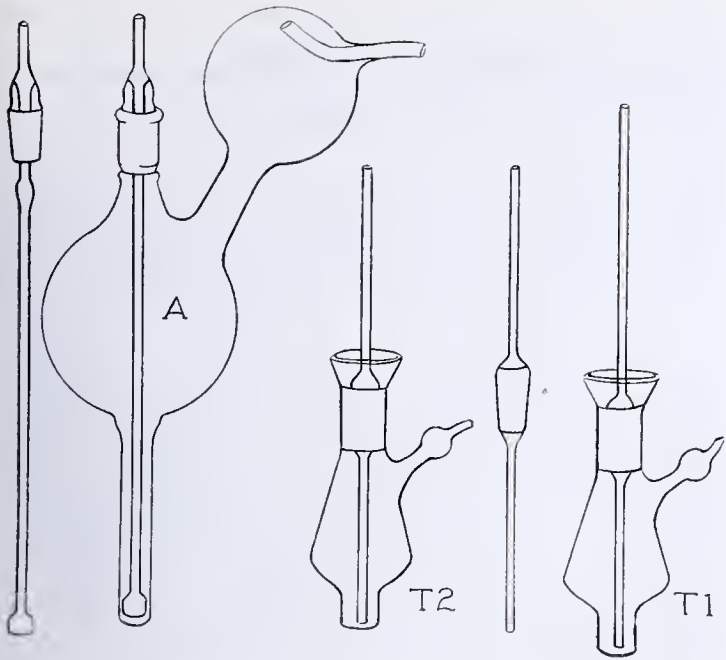


Figure 3. Aeration Flask and Reaction Vessels with Air-Inlet Tubes

action vessel and the vacuum stabilizing tank the meter was in a closed circuit and the air passing through it was at a reduced pressure, equal to 50 mm. (2 inches) of mercury at the aeration rate of 2.0 cubic feet in 40 minutes. The V.R.S. values obtained by aerating a sample with air metered at atmospheric pressure are higher than those with air metered at a vacuum equivalent to 50 mm. (2 inches) of mercury. Accordingly, the meter was kept at the air-intake end of the air-washing train and the flowmeters were calibrated against the meter with air passing through it at atmospheric pressure.

EFFECT OF RETORT PROCESSES ON V.R.S. CONTENT OF CANNED SARDINES (PILCHARDS). As a preliminary to the application of the method for the determination of spoilage in canned fish, it was deemed necessary to ascertain if any volatile reducing substances were formed during sterilization.

California sardines packed in No. 1 tall cans, natural style, were given the standard retort process of 90 minutes at 115.56° C. (240° F.). A number of cans were then subjected to additional processes up to a total of six. The last batch of cans, therefore, received a heating of 540 minutes or 9 hours at 240° F. All cans were cooled with water between individual cooks.

The extensive heat treatment had no significant effect on the content of volatile reducing substances in the canned sardines. Similarly, higher process temperatures do not influence the V.R.S. values.

Table I. Effect of Storage Temperature on Volatile Reducing Substances in California Sardines

Hours Stored	Microequivalents Reduction per 5 ML. of Press Juice of Fish			Sea Water at 37° F. (2.8° C.)		
	Raw	Canned	Organoleptic condition	Raw	Canned	Organoleptic condition
5	31.8	6.8	Passable	..	....	...
9	45.1	19.3	Raw not passable, canned, borderline	..	....	...
13	56.2	39.2	Not passable	..	....	...
17.75	123.3	73.0	Not passable	..	....	...
28	..	...	...	35.7	6.9	Passable
73	..	...	...	35.1	9.8	Passable
98	..	...	...	33.0	7.7	Passable

Fish at 80.6° F. were no longer fit for canning after 9 hours' storage, whereas fish at 37° F. remained fit for canning until the end of the experiment.

APPLICATION OF METHOD TO FISH. A large number of experiments have been performed on the progressive spoilage of various commercially canned Pacific Coast fish, such as sardines (pilchards), tuna, mackerel, and shad. The fish were examined in the raw state and after various canning operations, both organoleptically and for volatile reducing substances. Some representative data are presented in Tables I to V to illustrate the scope of the method and its usefulness as a means of evaluating progressive spoilage.

Table II. Volatile Reducing Substances Increase in Pacific Mackerel during Storage at 63.5° F. (17.5° C.)

Hours Stored	Microequivalents Reduction per 5 ML.		Condition of Raw Sample (Organoleptic Evaluation)
	Raw	Canned	
1.5	6.2	13.7	Passable
6.0	19.1	15.7	Slight sour odor, near borderline passability
8.8	19.6	19.2	Slight sour odor, near borderline passability
11.3	22.5	21.6	Sour odor, of doubtful passability
14.8	67.0	30.5	Not passable
31.3	163.5	164.0	Not passable

Table III. Volatile Reducing Substances Increase in Bluefin Tuna Canned after Various Storage Periods

Hours Stored	Microequivalents Reduction per 5 ML. of Canned Press Juice		Condition of Sample (Organoleptic Evaluation)
	Raw	Canned	
0	..	8.4	Passable
5.1	..	15.7	Passable
9.4	..	18.0	Passable
13.5	..	23.1	Doubtful passability
17.2	..	24.2	Not passable
21.5	..	34.8	Not passable

Table IV. Volatile Reducing Substances in Canned Atlantic Mackerel

Microequivalents Reduction per 5 ML.		Condition of Sample (Organoleptic Evaluation)
Raw	Canned	
14.4	..	Passable
24.5	..	Incipient spoilage, not passable
44.1	..	Putrid odor

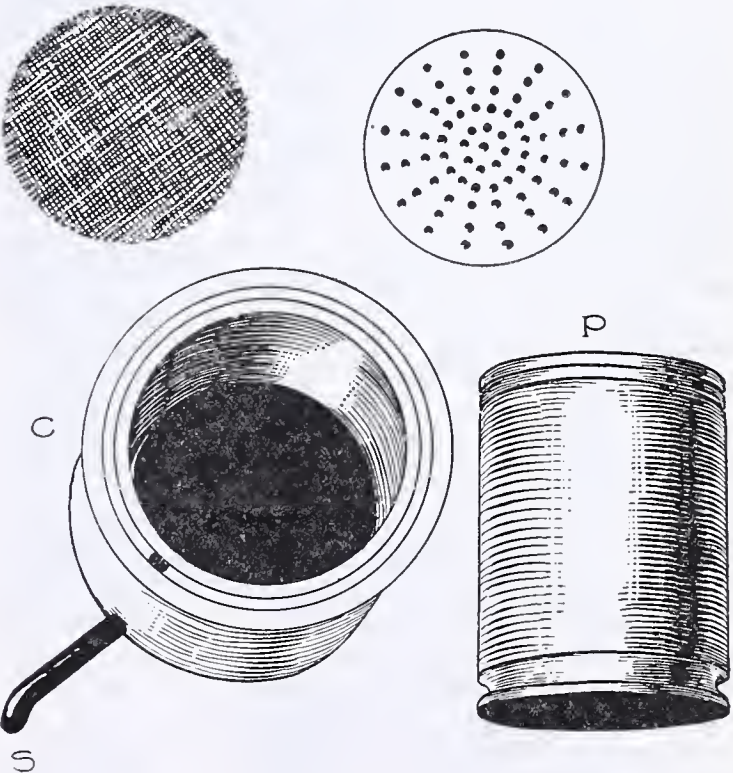


Figure 4. Apparatus for Obtaining Fish Press Juice  
Cylinder C, piston P perforated disk, and Chromel screen



APPLICATION OF METHOD TO OTHER FOODSTUFFS. Some experiments were conducted to ascertain the relationship between the content of volatile reducing substances and the state of preservation of foodstuffs other than fish.

Ground round steak was incubated in the original wrapping paper at 37.5° C. and periodically sampled as follows: A 5-gram sample was well triturated in a mortar with 10 ml. of redistilled water. The mixture was squeezed through cheesecloth and a 5-ml. aliquot of the press liquor was aerated with 2 cubic feet of air through two reaction vessels containing 10 ml. of oxidizing agent. The results obtained are shown in Table V.

Table V. Increase in Volatile Reducing Substances in Ground Round Steak during Storage at 37.5° C.

Hours Stored	Microequivalents Reduction	pH of Press Liquor	Organoleptic Condition
0	9.1	5.57	Normal meat odor
19	10.4	5.01	Normal meat odor
26	38.8	5.48	Strong, sour odor; meat has brown color; no longer appears edible
43	49.0	6.92	Definite tainted odor
50	57.4	7.33	Foul, putrid odor

Some preliminary experiments were also carried out on the increase in volatile reducing substances in eggs during storage and the relationship to the organoleptic condition. The results indicated the possible usefulness of the method for this commodity.

#### DISCUSSION

This method, in contrast to others proposed as measures of spoilage in such proteinaceous foodstuffs as fish and meat, is designed for determining only the volatile constituents which may be assumed to be responsible for the odors encountered. Thus is obtained a chemical and objective evaluation of the amount of odor which, when estimated organoleptically, is qualitative or at best only roughly quantitative and subjective.

The determination may be considered sensitive, since very dilute solutions of acetone and ethyl alcohol, from which no trace of the characteristic odors of the pure substances was detected, yielded relatively large reduction values. The sensitivity of the method in contrast to the organoleptic examination is exemplified by the comparison of odors emanating from fish liver oils. Two samples of oil yielded V.R.S. values of 102.5 and 108.6 microequivalents per 5 ml., respectively, while a third gave a reduction value of 46.1. Strong fishy odors of comparable intensity emanated from the first two samples, while the third had a milder fishy odor.

The total recovery of a particular volatile compound is of no immediate interest from a comparative point of view, since the percentage removal is practically constant for a given volume of air regardless of the original concentration. This suggests the adoption of an aeration volume more practical than that required for the complete removal of all volatile reducing substances. Accordingly, a volume of 2 cubic feet of air aspirated at the rate of 3 cubic feet per hour has been adopted. The same relationship between V.R.S. values in a series has been observed after aspirating 1 cubic foot of air through the sample as after 2 cubic feet. Therefore, if necessary the aeration may be stopped after 20 minutes when 1 cubic foot has been aspirated. For volatile reducing substances in press juices from fish which have undergone varying degrees of spoilage, 2 cubic feet of air aspirated at the rate of 3 cubic feet per hour removed between 75 and 90% of the amount removed by 6 cubic feet at the same rate.

The application of the method to problems concerned with the evaluation of spoilage is given in Tables I to V. The data presented in Table I may be of particular interest since the prolongation of keeping qualities through storage at lower temperatures

is shown. These experiments were carried out simultaneously with fish from the same boat load and catch.

It is evident that a definite trend or pattern is manifested in all fish varieties, both raw and canned, as spoilage progresses. The increase of volatile reducing substances in fish during deleterious storage is continuous and parallels that of organoleptic changes accompanied by the evolution of odorous substances. From an examination of data already accumulated on the increase of volatile reducing substances during spoilage of various fish species, ranges of values can be selected for fish of borderline or questionable passability and for those definitely not passable.

Biological variability with the resulting difficulty in obtaining an accurate and representative sample, and the pretreatments which the fish undergo during the canning process, all exert a marked influence upon the magnitude of the chemical evaluation in relation to the extent of spoilage. Here, as in other studies with biological materials, a considerable amount of data will tend to overcome these variabilities. Nevertheless a definite and progressive increase in V.R.S. values occurs in fish during prolonged storage under deleterious conditions. This increase, as may be expected, is somewhat less for the canned commodity, largely because of the volatilization of some of the gases through the exhaust or other pretreatment which the commodity undergoes during canning.

The accuracy of the method is not influenced by the type of spoilage. For instance, under one set of experimental conditions fish spoiled with the formation of sour, fatty acid odors, while under another set of conditions foul and putrid odors were produced. The specific products formed were different in these two experiments, yet both gave high values for volatile reducing substances and indicated advance degrees of spoilage.

The application of this aeration technique is not limited to spoilage determination. It has proved of value in estimating the concentration of volatile constituents in a variety of foods, where the amount of volatile material is used as an index of quality or desirability of a product.

#### SUMMARY

Based on the fact that proteinaceous foodstuffs emit odorous substances as they undergo spoilage during deleterious handling and storage, a method has been developed which determines these volatile materials and can be used to measure the state of freshness or the degree of spoilage in such foodstuffs.

A measured volume of washed air is aspirated through a suitably prepared sample of a material and through an alkaline potassium permanganate solution, all solutions being at room temperature. The amount of reduction of this oxidizing reagent is used as the measure of the concentration of the volatile substances.

The procedure has been applied particularly to determine state of freshness of raw and canned fish.

This method may be applied to the evaluation of odorous principles in other food commodities, where palatability and desirability are dependent upon the concentration of volatile substances.

#### ACKNOWLEDGMENT

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# Colorimetric Method for Determination of Ergosterol

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A quantitative colorimetric method for the estimation of pure ergosterol has been developed, as a modification of the reversed Salkowski reaction, using concentrated sulfuric acid and a carbon tetrachloride solution of ergosterol.

A SIMPLE, inexpensive method for the estimation of ergosterol has long been needed. The only satisfactory quantitative procedures which have been reported are those involving the use of a spectrograph, and this has limited their use to relatively few laboratories. It was, therefore, decided to adapt one of the well-known qualitative color reactions for sterols to a quantitative colorimetric procedure for the estimation of ergosterol. The first tried was the Rosenheim reaction (1) using trichloroacetic acid as the reagent. This was abandoned when it was found that the color increased in intensity progressively with time, making it very difficult to reproduce results. It was also noted that in the chloroform used as a solvent for this method there was a rather rapid autoxidation of the ergosterol, with the formation of a yellow color further interfering with the color developed.

It was then decided to try a modification of the "reversed Salkowski" reaction (2) using concentrated sulfuric acid. This proved to give a satisfactory, stable color, orange by transmitted light and having a strong green fluorescence. A carbon tetrachloride solution of ergosterol was used to avoid the autoxidative effects which were observed when chloroform was used as the solvent.

Table I. Per Cent Transmission

Mμ	20 min.	30 min.	40 min.	50 min.	60 min.
400	2	2	2	2	2
450	2	2	2	2	2
500	2	2	2	2	2
550	15.5	15	14.5	14.5	14.5
600	54	53.5	53	52.5	52
650	77.5	76.5	77	77.5	77

## EXPERIMENTAL

The Coleman Universal spectrophotometer Model 11 and its 13-mm. square tube cuvette were used in these studies.

Nissen and Petersen (3) have given a general discussion of the methods and problems of colorimetric studies.

**PURIFICATION OF ERGOSTEROL.** The ergosterol used was the "Puriss. in Nitrogen" grade prepared by the Glaxo Laboratories, Ltd., Greenford, Middlesex, England. This had turned yellow on standing, and it was necessary to recrystallize it by dissolving 50 grams in 400 ml. of hot, pure ethyl acetate and allowing it to crystallize overnight at 30° C. The product retained a slight yellow tinge, and the crystallization was repeated, resulting in a pure white product consisting of short needles. This was sucked free of solvent and dried at 30° C. under reduced pressure in a stream of nitrogen.  $(\alpha)_D^{25} = -126^\circ$ , found  $(\alpha)_D^{25} = -128.7^\circ$  (purified through benzoate) reported (1). Purity, approximately 98% (spectrograph).

The above observed rotation was the same as was obtained for the Glaxo ergosterol before it had yellowed and the specific rotation of the yellowed material was  $(\alpha)_D^{25} = -115.5^\circ$  before recrystallizations.

**SELECTION OF WAVE LENGTH FOR READING COLOR AND TIME OF COLOR DEVELOPMENT.** A 0.1% solution of ergosterol in pure, dry carbon tetrachloride was prepared and the color developed as follows: five milliliters of the solution were pipetted into each of five 25-ml. amber mixing cylinders and 10 ml. of concentrated sulfuric acid were added. These were mixed by inverting several times. The color formed in the lower acid layer, which was then read by pipetting this layer into the cuvette. A transmission curve for each sample was determined after they had stood for 20, 30, 40, 50, and 60 minutes, respectively (Table I).

Constancy of transmission is reached in about 40 minutes at 550 and 650 mμ.

A further check of the best wave length to be used was made by determining transmission curves as before where the concentration of ergosterol was varied in the range 0 to 0.1% with the color development time fixed at approximately 50 minutes (Table II).

The greatest reading differential in this concentration range is found at 550 mμ. It may therefore be expected that the greatest sensitivity will be obtained at this wave length.

A plot of the log transmission values obtained at 550 mμ against the concentration results in a smooth curve which is substantially a straight line in its middle portion. This portion was used in the remainder of the work, using a color development time of from 50 to 60 minutes.

Table II. Per Cent Transmission

Mμ	Ergosterol				
	0.02%	0.04%	0.06%	0.08%	0.10%
400	4	2	2	2	2
450	5	2	2	2	2
500	13.2	4.5	3	2.2	2
550	59	40	27.2	19.2	14
600	83.5	77	67.5	59	52.2
650	89.5	89	85	81	78

## FACTORS AFFECTING INTENSITY OF COLOR DEVELOPED

**ACID.** There are two ways in which the acid used might affect the color developed with ergosterol—the color of the acid and its concentration.

**Color.** Sulfuric acid is frequently discolored because of contact with the gasket material in the plastic closures. It is therefore important to refer all readings made to the particular acid used in developing the color.

**Concentration.** Several experiments were made to determine the effect of concentration variation in the acid used. Dilutions of 94, 90, and 86% were prepared and transmission-concentration curves determined as before (Table III).

The colors developed in this range by the 94 and 90% acids are practically identical, while those by the 86% are much darker. They also have a redder tinge, and tend to be cloudy, which may account, in part, for the decreased transmission values. In addition, a secondary blue-green color is developed in the carbon tetrachloride layer. It may be concluded, then, that acid from 90 to 94% will give consistent reproducible results. For this work, it was decided to fix the acid strength at 90% as a matter of consistency.

**AGING OF ERGOSTEROL SOLUTIONS IN CARBON TETRACHLORIDE.** When these solutions are allowed to stand, a change takes place even in the dark, resulting in a progressive increase in the color developed with sulfuric acid, even though there is no apparent change in the color of the solution as shown by Table IV. For this reason, solutions of ergosterol in carbon tetrachloride must be read as soon as they are prepared.

**SOLVENTS.** The question of the best solvent to be used in preparing ergosterol solutions has as yet not been thoroughly

Table III. Effect of Acid Concentration

% Ergosterol	% Transmission		
	94% acid	90% acid	86% acid
0.02	59.8	60.5	41.5
0.04	39.2	39.8	31
0.06	26.5	27	19.5
0.08	18.8	18.5	10
0.10	13.9	14.1	7



Table IV. Effect of Aging

% Ergosterol	% Transmission				
	0 days	1 day	2 days	7 days	28 days
0.10	14.1	9.8	...	1.0	Too dark
0.10	14.5	...	3.5	...	....

Table V. Per Cent Transmission

% Ergosterol	I	II	III	Average
0.01	..	78	77.2	77.6
0.02	59	60.5	61.5	60.3
0.03	..	49.5	48.7	49.1
0.04	40	39.8	39.5	39.8
0.05	..	31.5	32.0	31.7
0.06	27.2	27	26.8	27
0.07	..	21.5	21.6	21.5
0.08	19.2	18.5	18.8	18.8
0.09	..	15.5	15.6	15.5
0.10	14	14.1	13.9	14

studied. Carbon tetrachloride was used in this work, because ergosterol apparently does not autoxidize as readily in it as in chloroform. It is not at all impossible that other solvents such as cyclohexane or cyclohexanone, etc., would be useful.

#### TRANSMISSION-CONCENTRATION CURVE FOR ERGOSTEROL

Three separate 0.10% solutions of ergosterol in carbon tetrachloride were prepared and read immediately, using 90% sulfuric acid. In preparing the various concentrations of ergosterol,

the volume of carbon tetrachloride solution was maintained constant at 5 ml. Thus, in preparing the 0.02% solution, 1 ml. of the 0.10% solution was diluted in the mixing cylinder with 4 ml. of pure carbon tetrachloride, etc., then 10 ml. of 90% sulfuric acid were added to each by pipet and mixed by inverting five times. This mixing was repeated three more times at 10-minute intervals to maintain an even distribution of color. The total time from the addition of the sulfuric acid averaged about 50 minutes (Table V).

The per cent transmission (log) when plotted against the concentration results in a curve indicating obedience to Beer's law in the range 0.02 to 0.08% ergosterol. The reproducibility of the values is well shown by the close agreement obtained in the separate determinations. It is thus possible to determine an unknown quantity of pure ergosterol in a carbon tetrachloride solution by developing a color as above, reading the per cent transmission at 550  $m\mu$ , and applying this value to the standard curve, from which the concentration of ergosterol equivalent to the value may be read. By simple proportion, the fact that the standard ergosterol is but 98% pure may be compensated for, and the result reported in terms of 100% ergosterol.

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## Determination of Formaldehyde

### In the Presence of Acrolein and Other Aldehydes by the Polarographic Method

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Formaldehyde can be rapidly determined in the presence of acrolein and acetaldehyde by the polarographic method with an accuracy of  $\pm 2\%$ . The propionaldehyde wave does not overlap that of formaldehyde but its presence tends to cause low results unless determinations are run immediately. The most satisfactory results are obtained in 0.1 *N* lithium hydroxide containing 0.01 *N* lithium chloride, at constant temperature and constant pH, without removal of dissolved oxygen.

**A**LTHOUGH many methods are available for the determination of formaldehyde, little attention has been given to its determination in the presence of other aldehydes.

The Romijn potassium cyanide method (2, 4, 10, 11, 12) has been reported (1) to be suitable when acetaldehyde is present, if, in the procedure, the silver salt of the excess cyanide is immediately filtered off. According to Jahoda (6), however, this method is unsatisfactory for formaldehyde in the presence of acetaldehyde, and this investigator reports an accurate polarographic method. The polarographic method for this purpose has been confirmed by Grimaldi and Wells (5). Jonescu and Slusanschi (7) have worked out a time-precipitation relationship for the determination of certain aldehydes in aldehydic mixtures using dimethyldihydroresorcinol (dimedon) as a reagent. This method, however, is too time-consuming for industrial use.

This paper describes the determination of formaldehyde in the presence of acrolein by the polarographic method. The effect of the additional presence of acetaldehyde and propionaldehyde is also brought out.

It has been previously pointed out that in the polarographic determination of acrolein (9) in the presence of formaldehyde and

acetaldehyde, the formaldehyde wave occurs between two acrolein waves when working in an alkaline medium. Although there is an encroachment of the formaldehyde wave on the second acrolein wave, for the determination of acrolein such an encroachment offers no difficulty because the first acrolein wave gives reliable results. In the case of formaldehyde, however, this encroachment is important because this aldehyde gives only the one wave and its overlapping by the acrolein wave renders formaldehyde determinations inaccurate. Polarography, however, offers a reliable method since there is no overlapping when the concentration of acrolein does not appreciably exceed that of formaldehyde. When overlapping does occur, this may be corrected by repeating the determination with a much smaller sample.

A 0.1 *N* lithium hydroxide solution, 0.01 molar in lithium chloride, is most satisfactory for the determination of formaldehyde in the presence of acrolein within the limits stated above. No perceptible change in the pH occurs with dilution by the addition of the sample nor by traces of acid in the sample. The advantageous use of this solution confirms the previous report of Jahoda (6) that the use of a high pH made for greater sensitivity in the determination of formaldehyde in the presence of acetaldehyde.

#### SOLUTIONS

All indifferent salt solutions contained maxima inhibitor, consisting of 1 ml. of 0.2% methyl red alcoholic solution and 1.5 ml. of 0.02% bromocresol green alcoholic solution added per liter. They also contained 0.01 molar lithium chloride.



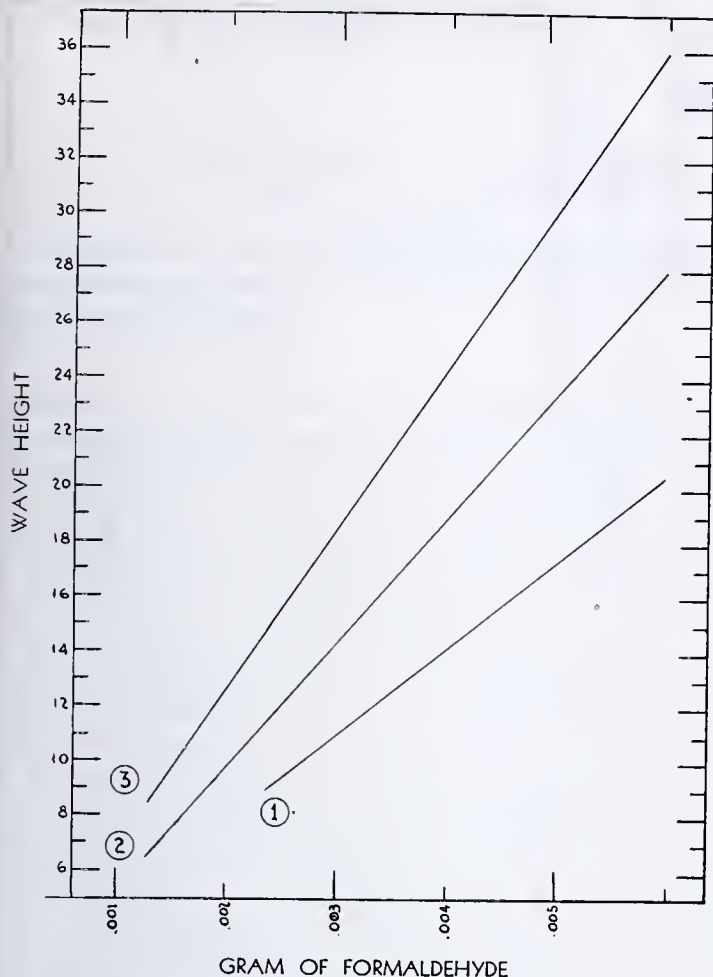


Figure 1. Effect of Temperature on Wave Height

Sensitivity 1/5. Volume 105 ml. pH 12.9  
Temperature (1) 20°, (2) 25°, (3) 30° C.

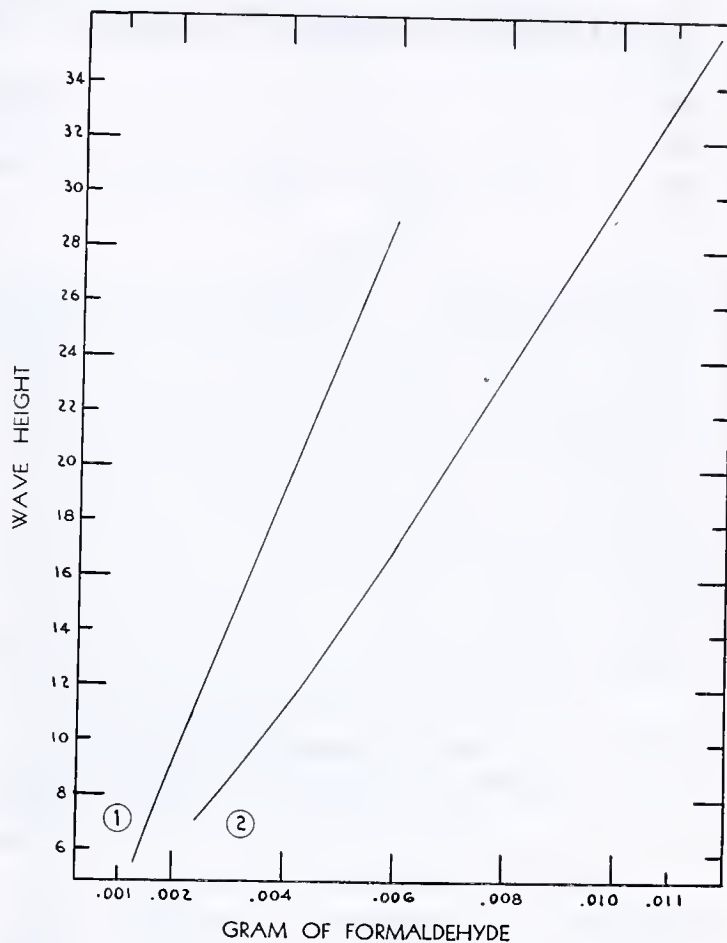


Figure 2. Effect of pH on Wave Height  
Sensitivity 1/5. Temperature 25.5° C. Volume 105 ml.  
1. pH 12.9. 2. pH 12.2

## ANALYSES

The polarograph used in this work was the Eledropode (manufactured by the Fischer Scientific Company, Pittsburgh, Pa.). All work was carried out at one fifth of the galvanometer sensitivity, using 125-ml. lipless beakers without removal of dissolved oxygen from the solutions. The removal of oxygen was not

Solution at pH 9.6 was prepared by adding carbon dioxide to a saturated solution of lithium carbonate.

Solution at pH 11.6 was a saturated lithium carbonate solution.

Solution at pH 12.2 was a 0.005 molar lithium hydroxide solution.

Solution at pH 12.9 was a 0.1 molar lithium hydroxide solution.

**ALDEHYDE SOLUTIONS.** The acrolein solution was prepared from commercial acrolein by collecting a 52° distillation fraction in water containing 0.01% hydroquinone as polymerization inhibitor. This solution was standardized by the Ripper bisulfite method as modified by Kolthoff and Furman (8). The formaldehyde solution was prepared by diluting Heyden's commercial formalin and analyzed by the hydrogen peroxide method (3).

The propionaldehyde was that of Eastman Kodak Company. All weighings were carried out in ampoules and the aldehyde analyzed by the Ripper bisulfite method.

Table I. Determination of Formaldehyde

Formaldehyde Present Mg.	Acetaldehyde Present Mg.	Formaldehyde Found Mg.	Error %
1.19	2.74	1.19	0.0
1.19	1.64	1.28	+7.5
1.19	1.09	1.19	0.0
1.19	0.55	1.19	0.0
2.38	0.55	2.38	0.0
2.38	1.09	2.40	+0.8
2.38 <sup>a</sup>	1.64	2.38	0.0
2.38	2.74	2.40	+0.8
3.56	0.55	3.45	-3.1
3.56	1.09	3.45	-3.1
3.56	1.64	3.45	-3.1
3.56	2.74	3.55	-0.3
5.94	5.48	5.63	-5.2
5.94	1.09	5.94	0.0
5.94	1.64	5.94	0.0
5.94	2.74	5.94	0.0

<sup>a</sup> See curve 2, Figure 4.

Table II. Determination of Formaldehyde

Formaldehyde Present Mg.	Acrolein Present Mg.	Formaldehyde Found Mg.	Error %
1.19	0.742	1.23	+3.4
1.19	1.48	1.21	+1.7
1.19	2.22	1.19	0.0
1.19	3.71 <sup>a</sup>	...	...
2.38	0.742	2.43	+2.1
2.38	1.48	2.34	-1.7
2.38	2.22	2.34	-1.7
2.38	3.71	2.38	0.0
3.56	0.742	3.51	-1.4
3.56	1.48	3.40	-4.5
3.56	2.22 <sup>b</sup>	...	...
3.56	3.71 <sup>b</sup>	...	...
5.94	0.742	5.94	0.0
5.94	1.48	5.94	0.0
5.94	2.22 <sup>b</sup>	...	...
5.94	3.71 <sup>b</sup>	...	...

<sup>a</sup> See curve 1, Figure 4.

<sup>b</sup> Acrolein wave overlaps that of formaldehyde.

Table III. Determination of Formaldehyde

Formaldehyde Present Mg.	Acrolein Present Mg.	Acetaldehyde Present Mg.	Formaldehyde Found Mg.	Error %
1.19	1.48	2.74	1.19 <sup>a</sup>	0.0
2.38	1.48	2.74	2.38	0.0
3.56	1.48	2.74	3.54	-0.6
5.94	1.48	2.74	5.72	-3.7
1.19	2.22	2.74	1.19	0.0
2.38	2.22	2.74	2.76 <sup>b</sup>	+16.0
3.56	2.22	2.74	3.34 <sup>b</sup>	-6.2
5.94	2.22	2.74	5.72 <sup>b</sup>	-3.9

<sup>a</sup> See curve 3, Figure 4.

<sup>b</sup> Waves overlap and determinations were calculated using apparent minimum between two waves.



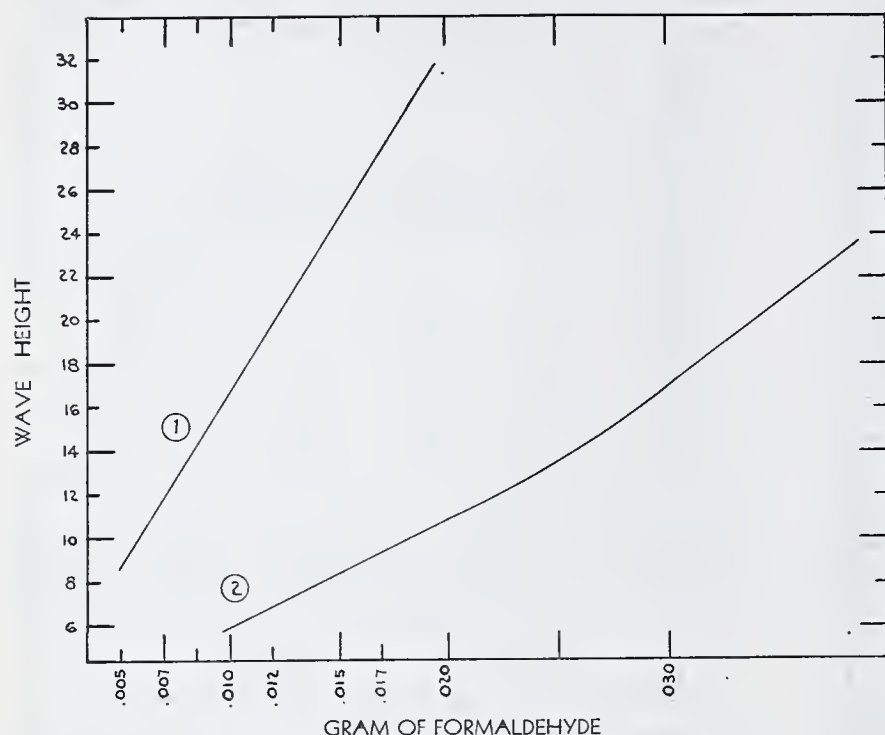


Figure 3. Effect of pH on Wave Height  
Sensitivity 1/5. Temperature 25.5° C. Volume 105 ml.  
1. pH 11.6. 2. pH 9.6

Table IV. Determination of Formaldehyde

Formaldehyde Present	Propionaldehyde Present	Formaldehyde Found	Error
Mg.	Mg.	Mg.	%
5.94	None	5.90	-0.6
5.94	2.48	5.51 <sup>a</sup>	-7.2
5.94	1.24	5.51 <sup>a</sup>	-7.2
5.94	6.20	5.42 <sup>a</sup>	-8.7
2.38	6.20	2.47 <sup>b</sup>	+3.7
2.38	6.20	2.06 <sup>c</sup>	-13.4
2.38	6.20	1.76 <sup>d</sup>	-26.0
2.38	2.48	2.36 <sup>b</sup>	-0.8

<sup>a</sup> Samples analyzed routinely, within 5 to 10 minutes.

<sup>b</sup> Samples run immediately on addition of propionaldehyde.

<sup>c</sup> 10-minute standing before analyzing.

<sup>d</sup> 15-minute standing before analyzing.

required, as no change in residual current was observed in the voltage increment used with the pH 12.9 lithium hydroxide base solution.

After addition of the sample to the base solution the cell can stand for at least 0.5 hour without loss of formaldehyde. This is an added advantage, in that one can prepare several cells without danger of loss in accuracy. Acetaldehyde and acrolein, however, must be determined immediately after adding the sample to the base solution.

For the most accurate quantitative work the temperature of the solution must be constant as observable by an ordinary laboratory thermometer.

The temperature effect on the polarographic wave is shown in Figure 1. The effect of pH on the polarographic wave is shown in Figures 2 and 3, which indicate the necessity of constant pH. Figure 4 shows typical polarographic curves taken on solutions containing both formaldehyde and acrolein.

#### RESULTS

A 0.1 *N* lithium hydroxide solution containing lithium chloride and maximum inhibitor giving a pH near 13 was selected as best for reproducibility, high sensitivity, and noninterference of acrolein and acetaldehyde in the determination of formaldehyde. The formaldehyde wave appears in the voltage range -1.50 to -1.65, having a corrected half-wave potential of -1.56 volts, a drop weight of 0.0045 gram, and at a drop rate of 6.3 seconds.

The determination is limited by the ratio of the quantities present in the aliquot taken for analysis. With 6 mg. of formaldehyde, no more than 0.75 mg. of acrolein may be present. However, with 1.2 mg. of formaldehyde, 2.2 mg. of acrolein do not

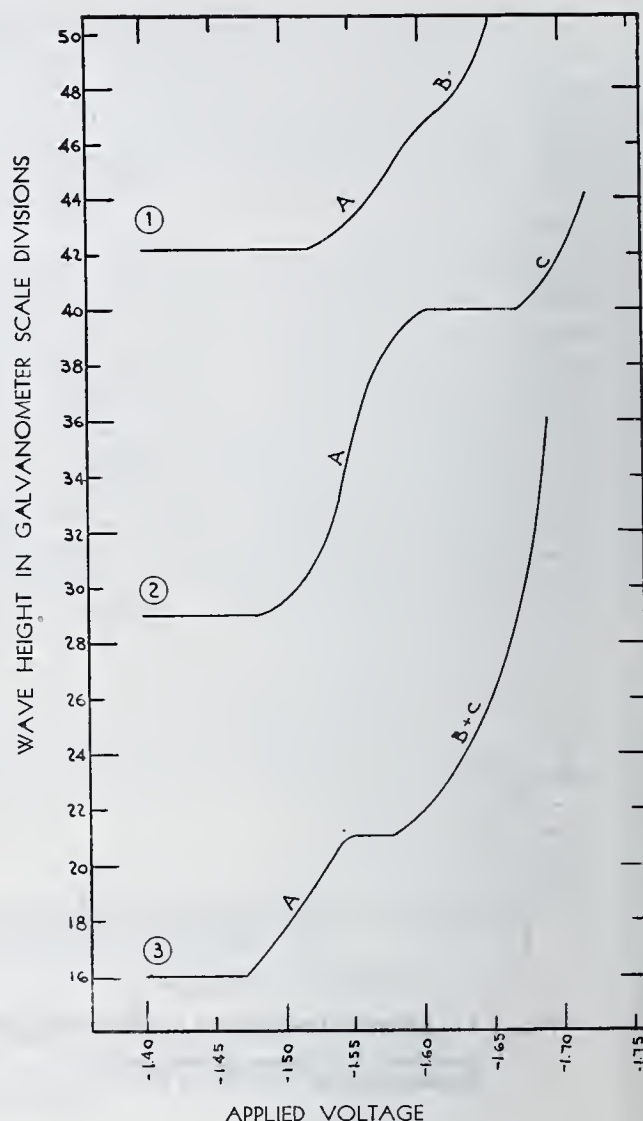


Figure 4. Current Voltage Curves for Formaldehyde  
Sensitivity 1/5. Volume 105 ml. pH 12.9. Temperature 25.5° C.  
A. Formaldehyde wave. B. Acrolein wave. C. Acetaldehyde wave  
1. 1.19 mg. formaldehyde, 3.71 mg. acrolein  
2. 2.38 mg. formaldehyde, 1.64 mg. acetaldehyde  
3. 1.19 mg. formaldehyde, 1.48 mg. acrolein, 2.74 mg. acetaldehyde

interfere. Acetaldehyde may be present up to 2.74 mg. The presence of propionaldehyde renders the formaldehyde determination low. If the sample is analyzed immediately after addition to the base solution the error is slight. The propionaldehyde wave occurs in the same voltage range as that of acetaldehyde and showed no overlapping with the formaldehyde wave.

The representative data shown in Tables I, II, III, and IV were obtained at pH 12.9, 25.5° C., and a galvanometer sensitivity of one fifth.

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# Analysis Data for the Ternary System Acetone-Benzene-Water

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A rapid and accurate method of analyzing mixtures containing acetone, benzene, and water has been developed, using density and refractive index data at 25° C.

THE need for a rapid and accurate method of analysis for mixtures containing acetone, benzene, and water arose in connection with solvent-recovery problems in pilot-plant-scale operations on the extraction of rubber from goldenrod leaves. The method described below has proved very practical and satisfactory. The fundamental data as well as the method should find applications in other investigations of solvent extraction or ternary liquid behavior involving this system.

The components of the system acetone-benzene-water are completely miscible at 25° C. for concentrations of acetone greater than 65 weight %; for lower proportions of acetone two phases may be formed when both benzene and water are present to any extent. Density and refractive index data for the acetone-benzene and acetone-water binary systems which are each mutually soluble have been determined by others over limited temperature ranges and are summarized in the International Critical Tables (7). More recently Ernst *et al.* (6) and Young (11) have reported additional density data on the acetone-water system at 25° and 20° C., respectively. Solubility curves and tie line data for the ternary system at 15°, 30°, and 45° C. were obtained by Briggs and Comings (4) by means of refractive index measurements. In the present investigation density and refractive index data at 25° C. for this system in the region of complete miscibility were obtained to permit analysis of mixtures in the homogeneous portion of the ternary diagram.

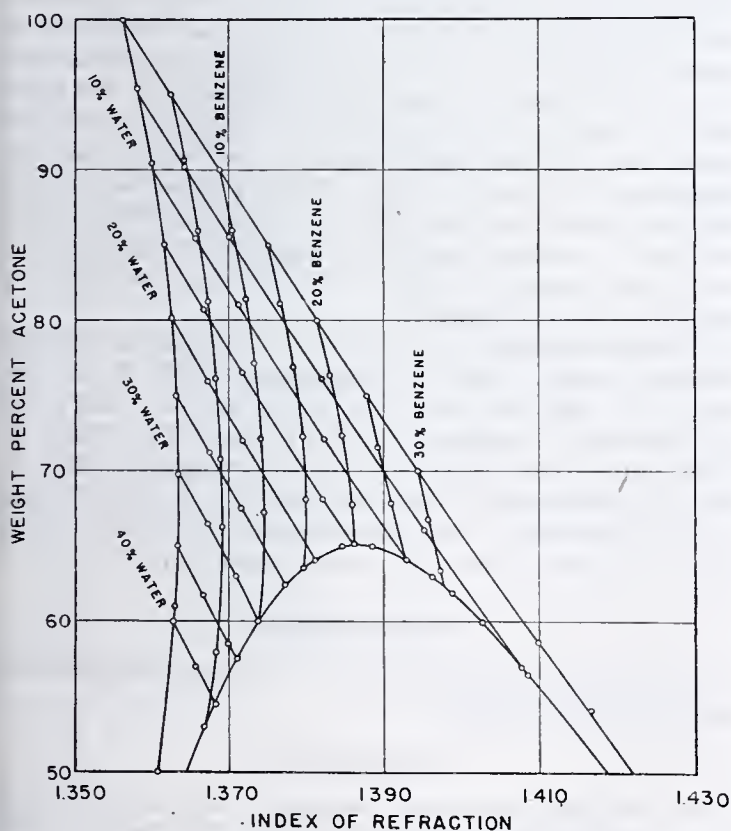


Figure 1. Refractive Indices for Mixtures of Acetone, Benzene, and Water

## EXPERIMENTAL

**MATERIALS.** Reagent grades of acetone and benzene were used without further purification. Densities and refractive indices of the original starting liquids at 25° C. were found to be as follows: acetone,  $d$ . 0.7857,  $n_D$  1.3562; benzene,  $d$ . 0.8731,  $n_D$  1.4972. These values agree sufficiently well with the better literature values summarized in the Annual Tables of Physical Constants (1) to permit analyses with an accuracy of 0.5% by the method herein described. Distilled water was used throughout.

Refractive index was determined with a dipping refractometer of precision  $\pm 0.00005$  on the solutions held in a metal cup which was surrounded by water circulated from a constant-temperature bath. Density was measured by National Bureau of Standards calibrated hydrometers having a precision of  $\pm 0.0002$ . For this determination approximately 250 cc. of the solutions were used in large stoppered test tubes immersed in the bath and were allowed to come to temperature equilibrium for at least 30 minutes before reading. The bath, set for 25° C., maintained the temperature constant to within  $\pm 0.05^\circ$  C.

Solutions were prepared by weighing out the components to 0.01 gram on a 2-kg. capacity analytical balance. Mixtures amounting to 300 cc. were prepared to give ample material for density measurement and to decrease inaccuracies due to evaporation of the more volatile components.

## TERNARY SYSTEM

The method of analyzing three component systems by measuring two independent properties has been described by Berl and Ranis (3) and others (2, 5, 8, 9, 10). In the present method re-

Table I. Experimental Data

Weight % Acetone	Weight % Benzene	Weight % Water	$n_D^{25}$	Density at 25° C.
100	.....	.....	1.3562	0.7857
94.96	5.04	.....	1.3625	0.7897
86.28	4.58	9.14	1.3654	0.8165
77.44	4.11	18.45	1.3671	0.8423
69.72	3.70	26.58	1.3675	0.8632
62.03	3.29	34.68	1.3666	0.8831
54.04	2.86	43.10	1.3650	0.9023
49.07	2.60	48.33	1.3634	0.9138
44.50	2.36	53.14	1.3617	0.9243
90.03	9.97	.....	1.3688	0.7938
82.07	9.09	8.84	1.3710	0.8194
73.75	8.17	18.08	1.3718	0.8441
66.21	7.33	26.46	1.3714	0.8650
80.13	19.87	.....	1.3812	0.8021
71.42	17.71	10.87	1.3823	0.8314
64.98	16.11	18.91	1.3814	0.8510
69.57	30.43	.....	1.3950	0.8107
66.89	29.26	3.85	1.3952	0.8208
64.84	28.36	6.80	1.3952	0.8286
54.16	45.84	.....	1.4166	0.8242
49.41	50.59	.....	1.4229	0.8282
40.61	59.39	.....	1.4350	0.8358
19.76	80.24	.....	1.4666	0.8543
.....	100	.....	1.4972	0.8731
95.44	.....	4.56	1.3581	0.7995
79.82	16.36	3.82	1.3784	0.8103
74.91	21.50	3.59	1.3850	0.8139
69.25	27.43	3.32	1.3927	0.8178
61.56	35.49	2.95	1.4029	0.8236
53.81	43.61	2.58	1.4145	0.8294
47.16	50.58	2.26	1.4233	0.8346
43.95	53.94	2.11	1.4288	0.8372
90.47	.....	9.53	1.3600	0.8143
81.53	9.88	8.59	1.3719	0.8192
73.60	18.65	7.75	1.3828	0.8236
80.14	.....	19.86	1.3626	0.8435
76.50	4.54	18.96	1.3678	0.8441
68.39	14.66	16.95	1.3795	0.8455
69.75	.....	30.25	1.3634	0.8704
67.92	2.63	29.45	1.3664	0.8702
65.75	5.74	28.51	1.3699	0.8696
60.98	.....	39.02	1.3629	0.8918
57.80	5.21	36.99	1.3686	0.8892
49.10	.....	50.90	1.3606	0.9181
48.10	.....	51.90	1.3600	0.9201
39.85	.....	60.15	1.3572	0.9366
39.77	.....	60.23	1.3573	0.9367
29.85	.....	70.15	1.3524	0.9541
21.47	.....	78.53	1.3477	0.9668
20.02	.....	79.98	1.3467	0.9702
.....	.....	100	1.3325	0.9970



Table II. Derived Data for Ternary Diagram at 25° C.

Refractive Index	Weight % Acetone	Weight % Benzene	Weight % Water	Refractive Index	Weight % Acetone	Weight % Benzene	Weight % Water
1.335	3.2	96.8	0.0	1.390	73.3	26.7	0.0
1.340	9.9	91.1	0.0		70.1	24.9	5.0
1.345	17.5	82.5	0.0		69.9	25.0	5.1
1.350	25.6	74.4	0.0		66.3	23.7	10.0
1.355	35.0	65.0	0.0		64.8 <sup>a</sup>	19.2	16.0
1.360	97.0	3.0	0.0	1.395	69.5	30.5	0.0
	93.7	1.3	5.0		68.6	30.0	1.4
	90.4	0.0	9.6		66.2	28.8	5.0
	47.9	0.0	52.1		63.5 <sup>a</sup>	27.5	9.0
	43.4 <sup>a</sup>	2.1	54.5	1.400	65.8	34.2	0.0
1.365	93.0	7.0	0.0		62.6	32.4	5.0
	89.6	5.4	5.0		61.4 <sup>a</sup>	31.5	7.1
	88.7	5.0	6.3	1.405	62.1	37.9	0.0
	86.0	4.0	10.0		59.0	36.0	5.0
	82.2	2.8	15.0		58.6 <sup>a</sup>	35.8	5.6
	77.9	2.1	20.0	1.410	58.5	41.5	0.0
	73.4	1.6	25.0		55.6 <sup>a</sup>	40.0	4.4
	68.5	1.5	30.0	1.415	55.0	45.0	0.0
	50.8 <sup>a</sup>	3.8	45.4		52.5 <sup>a</sup>	43.8	3.7
1.370	89.0	11.0	0.0	1.420	51.4	48.6	0.0
	87.0	10.0	3.0		49.3 <sup>a</sup>	47.7	3.0
	85.5	9.5	5.0	1.425	47.7	52.3	0.0
	81.9	8.1	10.0		46.2 <sup>a</sup>	51.2	2.6
	78.0	7.0	15.0	1.430	44.2	55.8	0.0
	73.6	6.4	20.0		43.1 <sup>a</sup>	54.9	2.0
	68.9	6.1	25.0	1.435	40.7	59.3	0.0
	63.8	6.2	30.0		37.3	62.7	0.0
	56.7 <sup>a</sup>	6.8	36.5	1.440	37.3	62.7	0.0
1.375	85.1	14.9	0.0		33.9	66.1	0.0
	81.6	13.4	5.0	1.445	33.9	66.1	0.0
	77.8	12.2	10.0		30.6	69.4	0.0
	73.8	11.2	15.0	1.450	30.6	69.4	0.0
	69.3	10.7	20.0		27.3	72.7	0.0
	64.5	10.5	25.0	1.455	27.3	72.7	0.0
	60.9 <sup>a</sup>	11.0	28.1		24.1	75.9	0.0
1.380	81.1	18.9	0.0	1.460	24.1	75.9	0.0
	77.6	17.4	5.0		20.7	79.3	0.0
	73.9	16.1	10.0	1.465	20.7	79.3	0.0
	69.8	15.2	15.0		17.4	82.6	0.0
	65.1	14.9	20.0	1.470	17.4	82.6	0.0
	63.7 <sup>a</sup>	15.2	21.1		14.0	86.0	0.0
1.385	77.1	22.9	0.0	1.475	14.0	86.0	0.0
	73.8	21.2	5.0		10.7	89.3	0.0
	71.1	20.0	8.9	1.480	10.7	89.3	0.0
	70.1	19.9	10.0		7.4	92.6	0.0
	65.8	19.2	15.0	1.485	7.4	92.6	0.0
	65.0 <sup>a</sup>	19.2	15.8		4.1	95.9	0.0
Density				1.490	4.1	95.9	0.0
0.790	98.4	0.0	1.6	1.495	0.8	99.2	0.0
	94.6	5.4	0.0				
0.800	95.2	0.0	4.8	0.850	77.7	22.3	0.0
	91.6	5.0	3.4		74.0	5.0	21.0
	88.0	10.0	2.0		70.5	9.5	20.0
	84.3	15.0	0.7		70.2	10.0	19.8
	82.6	17.4	0.0		66.3	15.0	18.7
0.810	91.8	0.0	8.2		64.7 <sup>a</sup>	17.7	17.6
	88.3	5.0	6.7		24.5	75.5	0.0
	84.6	10.0	5.4	0.860	73.9	0.0	26.1
	83.4	11.6	5.0		70.0	5.0	25.0
	80.9	15.0	4.1		66.1	10.0	23.9
	77.3	20.0	2.7		62.9 <sup>a</sup>	14.4	22.7
	73.7	25.0	1.3		13.5	86.5	0.0
	70.7	29.3	0.0	0.870	69.8	0.0	30.2
0.820	88.4	0.0	11.6		66.1	5.0	28.9
	84.4	5.6	10.0		62.0	10.0	28.0
	84.8	5.0	10.2		61.0 <sup>a</sup>	11.5	27.5
	81.2	10.0	8.8		2.9	97.1	0.0
	77.5	15.0	7.5	0.880	65.6	0.0	34.4
	73.8	20.0	6.2		62.0	5.0	33.0
	71.0	24.0	5.6		58.7 <sup>a</sup>	8.9	32.4
	70.2	25.0	4.8	0.890	61.7	0.0	38.3
	66.7	30.0	3.3		56.1 <sup>a</sup>	6.9	37.0
	63.1	35.0	1.9	0.900	57.4	0.0	42.6
	59.6	40.0	0.4		53.4 <sup>a</sup>	5.3	41.3
	58.6	41.4	0.0	0.910	52.8	0.0	47.2
0.830	84.9	0.0	15.1		50.3 <sup>a</sup>	3.8	45.9
	81.2	5.0	13.8	0.920	48.2	0.0	51.8
	77.6	10.0	12.4		46.6 <sup>a</sup>	2.8	50.6
	73.9	15.0	11.1	0.930	43.3	0.0	56.7
	71.1	18.9	10.0		42.2 <sup>a</sup>	1.9	55.9
	70.3	20.0	9.7	0.940	38.0	0.0	62.0
	66.8	25.0	8.2		37.3 <sup>a</sup>	1.1	61.6
	63.3	30.0	6.7	0.950	32.3	0.0	67.7
	59.5	35.0	5.5		26.1	0.0	73.9
	58.5	36.5	5.0	0.960	26.1	0.0	73.9
	56.1	40.0	3.9		19.5	0.0	80.5
	52.6	45.0	2.4	0.970	19.5	0.0	80.5
	47.3	52.7	0.0		12.3	0.0	87.7
0.840	81.3	0.0	18.7	0.980	12.3	0.0	87.7
	77.6	5.0	17.4		5.0	0.0	95.0
	74.0	10.0	16.0	0.990	5.0	0.0	95.0
	70.8	14.2	15.0				
	66.5	20.0	13.5				
	65.1 <sup>a</sup>	22.3	12.6				
	35.7	64.3	0.0				

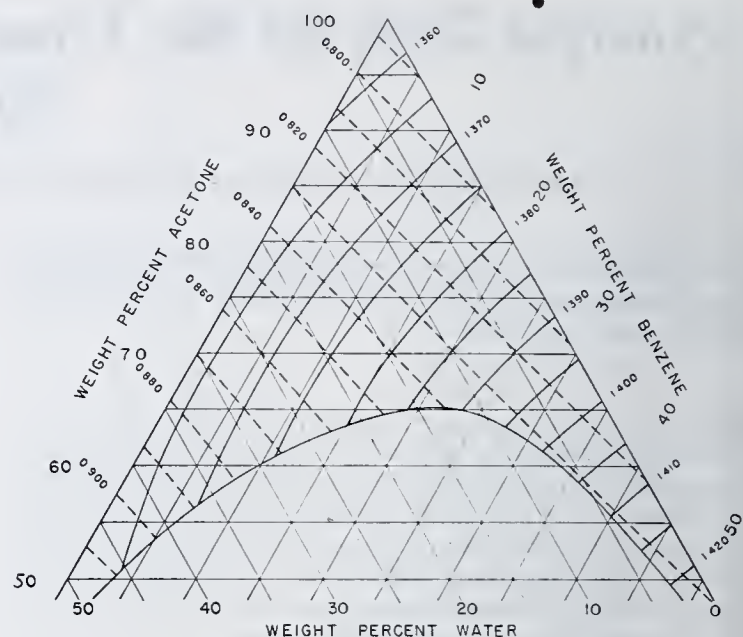
<sup>a</sup> Cloud point data.

Figure 2. Analysis Diagram for Acetone-Benzene-Water Mixtures Containing More Than 50% Acetone

fractive indices and densities were determined for a number of solutions of known composition, so that lines of equal density and of equal refractive index could be constructed. These lines were plotted on a triangular chart in such a way that the composition of an unknown could be found by interpolation of its measured density and refractive index values.

Two series of mixtures were prepared, one starting with acetone and water in different proportions, the other with acetone and benzene; the third component was added stepwise until the cloud point was reached, measurements of refractive index and density being made after each addition. The data of the one series thus overlapped and served to check the data of the other over most of the region of homogeneous mixture. Table I includes experimental data for the ternary system at 25° C.

From graphical interpolation of these data it was possible to construct plots of the refractive index *vs.* acetone composition for various mixtures of constant water or constant benzene contents as shown in Figure 1. Using values obtained from this figure lines of common refractive index were constructed on the triangular chart for the ternary diagram. The density lines were determined in a similar manner. Figure 2 shows both sets of lines for ternary mixtures containing more than 50% acetone. Data used to construct the curves in Figure 2 and additional data covering the system for mixtures with less than 50 weight % acetone are given in Table II.

If the density and refractive index of a homogeneous unknown of acetone, benzene, and water are determined at 25° C., the composition in weight per cent of the sample may be found to the nearest 0.5% by interpolation of data in Figure 2 and Table II.

Cloud-point compositions at 25° C. were compared to data obtained by interpolation of values reported by Briggs and Comings (4) at 15° and 30° C. Good agreement between these two sets of data was observed over the entire solubility curve.

#### ACKNOWLEDGMENT

The help and suggestions of Evald L. Skau of this laboratory are gratefully acknowledged.

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## Analysis of Cellulose Derivatives

### Total Acyl in Cellulose Organic Esters by Saponification in Solution

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Most saponification methods for the determination of total acyl in cellulose organic acid esters involve heterogeneous conditions. A method has been developed in which the sample is saponified in solution. As a result, homogeneous saponification conditions exist which eliminate errors due to the condition of the sample, improve the accuracy, shorten the time of saponification, and give a better end point. The effects of time, temperature, and alkalinity were studied and the optimum conditions for each were established. The range of applicability of this method is discussed and compared with the Eberstadt and alcoholic alkali methods. The basic principle followed involves solution of the sample in a suitable solvent, followed by stepwise additions of alkali and water under conditions such that the ester remains in solution until saponification is practically complete. The regenerated or only slightly esterified cellulose

finally precipitates in a soft finely divided form which does not interfere with completion of the reaction or the back-titration. The precision obtainable by this method has been evaluated and limits of uncertainty (maximum range within which nearly all carefully run values should fall) are  $\pm 0.1$  to  $0.2\%$  acetyl, depending on the type of ester being analyzed. A measure of the accuracy of the method was obtained by analyzing samples for free hydroxyl and for acetyl or apparent acetyl (saponification value calculated as acetyl) and complete acyl in the case of cellulose mixed esters. The observed acetyl or apparent acetyl values were compared with those calculated by difference from observed free hydroxyl contents and molar ratios of the acyl groups in the case of cellulose mixed esters, assuming exactly 3 hydroxyls per glucose unit of cellulose. The agreement was well within experimental error.

THE determination of total acyl in cellulose organic esters by saponification in solution overcomes some of the difficulties encountered in the heterogeneous saponification methods such as the Eberstadt (or Knoevenagel) and the alcoholic alkali methods described in the first paper of this series (5). A solution method has been developed which eliminates the effect of the physical form of the sample, permits the use of a lower alkalinity and a shorter time of saponification, and gives a more satisfactory end point and a slight improvement in accuracy. However, the solvents must be varied to suit the composition and solubility of each type of ester, and the manipulation is somewhat involved and must be varied for each different type of ester. It is thus better adapted to routine analyses of familiar samples than to isolated analyses of unknown samples.

The basic principle followed involves solution of the sample in a suitable solvent, followed by stepwise additions of alkali and water under conditions such that the ester remains in solution until saponification is practically complete. When the regenerated or only slightly esterified cellulose finally comes out of solution it is in a soft finely divided form which does not interfere with the completion of the reaction or the back-titration. Conditions of temperature and alkali concentration are chosen to ensure a complete reaction and to avoid the formation of acids by the decomposition of cellulose.

At least three saponification methods are now available for the analysis of the organic esters of cellulose. The Eberstadt method is simple and best adapted to the acetone-soluble cellulose acetates and to hydrolyzed cellulose acetate propionates and acetate butyrates having low propionyl and butyryl contents, if these samples are in good physical form. It is also recommended for cellulose acetates having less than  $15\%$  acetyl. The alcoholic alkali method is simple and is widely applicable to cellulose esters, but usually is less accurate than the Eberstadt method. Both methods are inaccurate when the liquid medium used

swells and softens the ester excessively but does not dissolve it. The saponification in solution method described in this paper may be used on solvent-soluble cellulose acetates, propionates, butyrates, acetate propionates, and acetate butyrates. It is particularly useful for esters not readily analyzable by the Eberstadt method and where good accuracy is desired.

There are several references in the literature to methods in which the cellulose ester is dissolved in a solvent, such as acetone or pyridine, and the alkali added dropwise. Partial saponification is thus carried out in solution, but no precautions are given for maintaining a solvent system and for holding the saponifying ester in solution as long as possible.

Barnett (1) described a procedure, applied only to acetone-soluble cellulose acetates, by which a 0.3-gram sample was dissolved in 30 ml. of acetone and was saponified with 50 ml. of  $0.1 N$  sodium hydroxide for 24 hours at room temperature. After diluting with water, the excess alkali was back-titrated with  $0.1 N$  acid using phenolphthalein indicator. It was necessary to run a blank on the reagents and on cellulose and a considerable correction was applied. Battegay and Penche (2) analyzed cellulose acetate by dissolving a 0.3 to 0.5-gram sample in 30 ml. of pyridine at  $40^\circ C$ . The solution was cooled to  $25^\circ C$ , and 50 ml. of  $0.5 N$  alkali were added. After shaking for 0.5 hour the excess alkali was back-titrated. Murray, Staud, and Gray (9) developed a rapid acetyl method in which a 0.5-gram sample was dissolved in 20 ml. of pyridine and was saponified for 0.5 hour at about  $50^\circ C$ . with 20 ml. of  $0.5 N$  alkali. This method is rapid and applicable to a certain range of cellulose acetates, but the temperature used is too high. Charriou and Valette (3) determined the acetyl in cellulose acetates by dissolving a 1.5-gram sample in 100 ml. of acetone and adding 50 ml. of  $0.5 N$  sodium hydroxide dropwise with agitation. The flask was stoppered and agitated vigorously for 0.5 hour and the excess sodium hydroxide titrated with  $0.5 N$  sulfuric acid using phenolphthalein indicator. A Du Pont specification (4) gives a method in which a 1.5-gram sample is dissolved in 100 ml. of acetone, 50 ml. of  $0.5 N$  sodium hydroxide are added dropwise, and after 3 hours of agitation the excess is titrated with  $0.5 N$  hydrochloric acid using phenolphthalein indicator.

<sup>1</sup> Now in service of U. S. Coast Guard.



In earlier experiments in this laboratory (10) various solvents were tried including acetone, acetone-water, and acetone-alcohol mixtures with aqueous alkali at various times and temperatures. Each of these methods is applicable to a narrow range of cellulose esters. In some cases a temperature of 40° to 55° C. was used, but this does not give satisfactory results. Side reactions occur which require exact control of the time to obtain accurate results. Because of these limitations and their specific character these methods have not been widely accepted.

#### PROCEDURE

The more common cellulose lower fatty acid esters have been divided into the following groups, based on their solubility differences, and variations made in the procedure to meet the requirements of each:

GROUP I. Solvent-soluble cellulose acetates having about 15 to 30% acetyl, which require water in the solvent mixture to effect solution of the sample. These solutions tolerate a considerable amount of water during saponification.

GROUP II. Cellulose acetates having 30 to 44.8% acetyl, most cellulose acetate propionates and acetate butyrates having less than 30% propionyl or butyryl, and similar esters whose solutions will tolerate some water.

GROUP III. Cellulose propionates, butyrates, acetate propionates, acetate butyrates, etc., having 30 to 45% propionyl or butyryl, which are very water-resistant, and the solutions of which tolerate little water.

GROUP IV. Cellulose propionates, butyrates, and mixed esters having more than 45% propionyl or butyryl whose solutions will tolerate practically no water.

The samples are dried about 2 hours at 100° to 110° C., cooled in a desiccator, and accurately weighed into 250-ml. glass-stoppered Erlenmeyer flasks. Each sample is dissolved in a solvent mixture which meets the following requirements: It must not react with the alkali, must tolerate the addition of considerable amounts of water, preferably should be a solvent for a wide range of cellulose esters, and the resulting system must be a solvent for the alkali. Blanks are run on each solvent combination used, and are carried through all steps of the manipulations

In the procedure variations for the four groups, solvent mixtures are specified which have been used successfully. Definite additions of water and alkali are also recommended. Other solvents meeting the requirements listed above and other amounts and orders of addition of water and alkali should yield satisfactory results if the additions are made at a rate consistent with the rate of saponification and change in solubility, so the steadily changing ester will remain in solution as long as possible. Enough alkali must be added at the start to produce appreciable diminution in acyl content, and before precipitation occurs enough water must be added to maintain a solution. More or perhaps all of the alkali can then be added, together with enough water to produce the desired dilution. If the manipulations are carried out properly, the partly esterified or regenerated cellulose separates slowly from solution and settles in a soft bulky form. If the precipitate is coarse or gummy the analysis is probably spoiled and should be repeated. In general, a total of 120 ml. of solvent and 30 ml. of alkali is added, which produces a dilution of the 0.5 *N* alkali to 0.1 *N*.

GROUP I. A 1-gram sample is dissolved in 50 ml. of the solvent mixture. In the case of cellulose acetates in the lower acetyl range of this group a 2:1:1 by volume mixture of water, pyridine, and acetone is recommended, while in the upper acetyl range of this group the same solvents in a ratio of 1:1:1 are suggested. During saponification 70 ml. of water (making a total of 120 ml. of added solvent) and 30 ml. of 0.5 *N* alkali are added. The distilled water is added to the solution with vigorous shaking until a temporary precipitate is observed at the point where the water first comes in contact with the solution or until the entire 70 ml. have been added. A 15-ml. portion of alkali is added, or alkali is added until a temporary precipitate forms. Then the remainder, if any, of the 70 ml. of water is added and the flask shaken until the solution becomes turbid. Finally alkali is added to give a total of 30 ml. The flasks are stoppered and allowed to stand at room temperature for 6 hours or more.

GROUP II. A 0.5-gram sample is dissolved in 50 ml. of a 1:1 by volume mixture of pyridine and acetone. A 20-ml. portion of distilled water and 10 ml. of 0.5 *N* alkali are added with swirling, which is continued until a slight turbidity appears. Then the remaining 50 ml. of distilled water and 20 ml. of alkali are added. Six hours or more at room temperature are allowed for saponification.

GROUP III. A 0.5-gram sample is dissolved in 100 ml. of a 1:1 by volume mixture of acetone and methyl alcohol. A 10-ml. portion of 0.5 *N* alkali is added slowly with swirling, until a definite turbidity develops. The remaining 20 ml. of alkali and 20 ml. of water are then added slowly. Six hours or preferably overnight at room temperature must be allowed for saponification.

GROUP IV. A 0.5-gram sample is dissolved in 100 ml. of a 1:1 by volume mixture of pyridine and methyl alcohol. A 30-ml. portion of 0.5 *N* methyl-alcoholic alkali is added slowly with swirling. A 20-ml. portion of water is added slowly and the flask swirled until the solution becomes turbid, so that the regenerated cellulose will settle in a finely divided form. The flasks are allowed to stand overnight at room temperature for completion of saponification.

In all cases, the excess of alkali is back-titrated using standard 0.5 *N* hydrochloric acid and phenolphthalein indicator.

The result may be calculated as follows:

$$\frac{\left[ \left( \text{Ml. of HCl for blank} \right) - \left( \text{ml. of HCl for sample} \right) \right] \times \text{HCl normality} \times 4.3}{\text{sample weight}} = \% \text{ apparent acetyl}$$

This result is given in terms of per cent acetyl in the case of cellulose acetate and as apparent acetyl (saponification value calculated as acetyl) in the case of other esters.

#### STUDY OF THE METHOD

The effects of some of the variables of the method were studied using four samples, one representative of each of the four groups described. The analyses of these samples are given in Tables I,

Table I. Effect of Time and Temperature of Saponification

Sample	Time Hours	Temperature of Saponification— Acetyl or apparent acetyl				
		0° C. %	20–35°C. %	40° C. %	50–55° C. %	
Low-acetyl cellulose acetate 16.8% acetyl	1	16.0	16.4	...	16.3	...
		16.0	16.4	...	16.4	...
	3	16.1	16.5	...	16.8	...
		16.2	16.5	...	16.9	...
	6	16.2	16.8	...	16.9	...
		16.2	16.8	...	17.0	...
	16	16.2	16.8	16.8	17.6	18.5 <sup>a</sup>
		16.2	16.8	16.8	17.8	18.7 <sup>a</sup>
	24	16.3	16.8	...	16.3	17.8 <sup>a</sup>
		16.4	16.8	...	16.5	17.9 <sup>a</sup>
Acetone-soluble cellulose acetate 40.4% acetyl	1	39.7	40.0	...	39.8	...
		39.8	40.0	...	39.9	...
	3	40.0	40.1	...	40.3	...
		40.0	40.0	...	40.3	...
	6	39.9	40.4	...	40.7	...
		40.0	40.4	...	40.7	...
	16	40.0	40.4	40.4	39.2	43.3 <sup>a</sup>
		40.0	40.5	40.5	39.3	43.4 <sup>a</sup>
	24	40.1	40.4	...	36.0	43.3 <sup>a</sup>
		40.1	40.5	...	36.1	43.5 <sup>a</sup>
Cellulose acetate butyrate I 35.0% apparent acetyl 12.6% acetyl 37.0% butyryl	1	22.0	32.0	...	34.5	...
		22.2	32.1	...	34.5	...
	3	22.2	34.7	...	35.3	...
		22.3	34.7	...	35.4	...
	6	28.1	35.0	...	35.8	...
		28.2	35.0	...	35.9	...
	16	33.0	34.9	35.4	37.6	39.2 <sup>a</sup>
		33.1	35.0	35.5	37.6	39.2 <sup>a</sup>
	24	34.5	35.0	...	34.0	39.7 <sup>a</sup>
		34.5	35.0	...	34.1	39.7 <sup>a</sup>
Cellulose acetate butyrate II 34.4% apparent acetyl 0.7% acetyl 55.6% butyryl	1	4.4	18.5	...	31.9	...
		4.5	18.7	...	32.0	...
	3	4.6	25.7	...	35.1	...
		4.6	25.8	...	35.1	...
	6	10.5	33.5	...	35.4	...
		10.7	33.8	...	35.5	...
	16	17.8	34.3	34.6	38.5	...
		17.9	34.4	34.6	38.7	...
	24	21.3	34.4	...	39.3	...
		21.3	34.4	...	39.4	...
	48	30.6	34.4	36.1	42.7	...
		30.8	34.4	36.0	42.9	...

<sup>a</sup> Calculations made using titration figure for unheated blank.



II, and IV; two are cellulose acetates and two cellulose acetate butyrates, the last of which is essentially a butyrate with only a very little acetyl.

In these experiments good commercial grades of methyl alcohol and acetone were used. A good commercial grade of pyridine was treated with flake sodium hydroxide and fractionated. The water azeotrope was removed and the fraction taken which boiled at 115.5° to 116.0° C.

**EFFECT OF TIME AND TEMPERATURE OF SAPONIFICATION.** The effects of the time and temperature of saponification were studied as shown in Table I. The above procedure was followed with these two variations.

These data show that better results are obtained at the room temperature range (20° to 35° C.) than at 0°, 40°, or 50° to 55° C. In the case of cellulose acetates and cellulose acetate propionates or butyrates of low propionyl or butyryl content, acceptable results may be obtained after 6 hours at room temperature which do not change even after 48 hours. About 16 hours at this temperature must be allowed for cellulose acetate propionates or acetate butyrates having a high propionyl or butyryl content.

At 0° C. the reaction is very slow and in most cases is not complete even after 48 hours. Erratic results are obtained at 50° to 55° C. Changes in the titer of the blank, as a result of heating, indicate a side reaction. There is a considerable reaction after 16 hours, and as much as 2 ml. of alkali was consumed in some of the blanks. Results are too high when calculated using the titration figure of an unheated blank. There is also some side reaction in 48 hours at 40° C. After 16 hours there has been a reaction in some cases, and results were acceptable in other cases. Because of this erratic behavior, saponification temperatures should be kept below 40° C.

**EFFECT OF TIME OF SAPONIFICATION AND ALKALI CONCENTRATION.** The effects of the time of saponification and the alkali

Table II. Effect of Time and Alkali Concentration

Sample	Time Hours	Alkali Concentration		
		0.05 N	0.1 N	0.2 N
		Acetyl or %	Apparent Acetyl %	Acetyl %
Low-acetyl cellulose acetate, 16.8% acetyl	1	15.9	16.4	16.8
		15.9	16.4	16.8
	3	16.1	16.4	16.8
		16.2	16.5	16.8
	6	16.4	16.8	16.8
		16.4	16.8	16.8
	16	16.3	16.8	16.8
		16.4	16.8	16.8
	24	16.4	16.8	16.8
		16.4	16.8	16.8
Acetone-soluble cellulose acetate, 40.4% acetyl	1	16.5	16.8	16.8
		39.6	40.0	40.4
		39.7	40.0	40.4
	3	39.9	40.0	40.4
		40.0	40.1	40.4
	6	40.2	40.4	40.4
		40.2	40.4	40.4
	16	40.2	40.4	40.4
		40.2	40.5	40.4
	24	40.2	40.4	40.4
Cellulose acetate butyrate I 35.0% apparent acetyl 12.6% acetyl 37.0% butyryl		40.3	40.5	40.4
	48	40.3	40.4	40.4
		40.3	40.4	40.4
	1	27.0	32.0	34.7
		27.2	32.1	34.8
	3	30.4	34.7	35.0
		30.6	34.7	35.0
	6	34.1	35.0	35.0
		34.1	35.0	34.9
	16	34.7	34.9	35.3
Cellulose acetate butyrate II 34.4% apparent acetyl 0.7% acetyl 55.6% butyryl		34.8	35.0	35.3
	24	35.0	35.0	35.3
		35.0	35.0	35.4
	48	35.0	35.0	35.4
		35.1	35.0	35.5
	1	8.2	18.5	26.6
		8.4	18.7	26.8
	3	13.1	25.7	31.9
		13.3	25.8	32.0
	6	22.1	33.5	34.3
		22.3	33.8	34.4
	16	24.8	34.3	31.0
		24.9	34.4	31.2
	24	31.7	34.4	30.7
		31.9	34.4	30.5
	48	34.1	34.4	32.6
		34.2	34.4	32.4
				34.1
				34.2
				34.0
			34.2	
			32.8	
			32.5	

Table III. Precision Studies

Test No.	Per Cent Acetyl or Apparent Acetyl			
	Low-acetyl cellulose acetate	Acetone-soluble cellulose acetate	Cellulose acetate butyrate I	Cellulose acetate butyrate II
1	16.80	40.40	34.96	34.42
2	16.80	40.40	34.99	34.36
3	16.79	40.42	34.90	34.50
4	16.79	40.42	34.96	34.42
5	16.76	40.44	34.98	34.48
6	16.77	40.41	34.94	34.37
7	16.87	40.40	35.05	34.35
8	16.86	40.49	34.98	34.40
9	16.86	40.40	35.01	34.51
10	16.87	40.38	35.07	34.30
Numerical average = $\bar{X}_1$				
$\bar{X}_1 = 16.81$ 40.41 34.98 34.41				
Sum of squares of deviations from average = $\Sigma d^2$				
$\Sigma d^2 = 0.0173$ 0.0088 0.023 0.042				
Standard deviation = $\sigma_{10} = \sqrt{\frac{\Sigma d^2}{10}}$				
$\sigma_{10} = 0.042$ 0.030 0.048 0.065				
Limit of uncertainty of average = $LU_{av.} = \bar{X}_1 \pm \frac{3\sigma_{10}}{\sqrt{10}}$				
$LU_{av.} = 16.77$ to $16.85$ 40.38 to $40.44$ 34.93 to $35.03$ 34.35 to $34.47$				
Limit of uncertainty of individual result = $LU_1 = \pm \frac{3\sigma_{10}}{0.923}$				
$LU_1 = \pm 0.13$ $\pm 0.10$ $\pm 0.16$ $\pm 0.21$				

concentration were studied as shown in Table II. The nominal concentration is that produced by dilution of the added alkali by the solvents added. The actual concentration is probably below this value at all times.

These data show that satisfactory results are obtained when 0.5 N alkali, diluted by the solvents added to produce a concentration of 0.1 N, is used. In this case the reaction is complete in from 6 to 16 hours. The results obtained during this time are not changed even after 48 hours.

When 0.25 N alkali, diluted by the solvents to 0.05 N, is used the reaction is very slow, and in most cases is not complete after 48 hours. In the case of 1.0 N alkali diluted to 0.2 N, acceptable results are obtained in one hour on cellulose acetates, and this value remains constant for 48 hours. The reaction is complete in the case of cellulose acetate butyrate I after 3 hours, but the results are too high after about 16 hours. Results on cellulose acetate butyrate II are very erratic. When 2.5 N alkali is used to give a nominal concentration of 0.5 N after dilution, suitable additions of alkali cannot be made. The alkalinity is so high that the sample precipitates immediately, giving unsatisfactory results.

#### PRECISION STUDIES

The precision attainable by this procedure was evaluated by the method described by Moran (8). The same four samples used in the above studies were analyzed ten times by the same operator under the most favorable conditions, and the data obtained are shown in Table III. The average of the ten values ( $\bar{X}_1$ ) was calculated for each sample, individual variations from the average were squared, and the sums of these squares ( $\Sigma d^2$ ) then used in the following calculations:

Standard deviation ( $\sigma$ ) is the most accurate measure of dispersion about an arithmetical mean, and mathematically is the square root of the average of the squares of the individual deviations:

$$\sigma_{10} = \sqrt{\frac{\Sigma d^2}{10}}$$

The limit of uncertainty of the average ( $LU_{av.}$ ) is the narrowest range within which any one result can be guaranteed:

$$LU_{av.} = \bar{X}_1 \pm \frac{3\sigma_{10}}{\sqrt{10}}$$

The limit of uncertainty of the method under the best possible conditions ( $LU_1$ ) is the precision range within which a high proportion of results (997 out of 1000) should fall when good samples are analyzed by a skilled technician working under closely controlled conditions:



Table IV. Test of Accuracy

Sample	Low-Acetyl Cellulose Acetate	Acetone-Soluble Cellulose Acetate	Cellulose Triacetate	Cellulose Acetate Butyrate I	Cellulose Acetate Butyrate II
Acyl content					
% acetyl	16.8	40.4	44.4	12.6	0.7
% butyryl	...	...	...	37.0	55.6
Per cent free hydroxyl observed	18.16 <sup>a</sup>	3.03	0.15	2.20	0.15
Hydroxyl groups per glucose unit					
Esterified	0.757	2.513	2.955	Acetyl Butyryl 0.927 1.650	0.059 2.853
Free, observed	2.070	0.472	0.025	0.410	0.032
Observed, total	2.827	2.985	2.980	2.987	2.944
Apparent acetyl					
Observed	16.8	40.4	44.4	35.0	34.4
Calculated from hydroxyl and molar ratio	18.9 <sup>b</sup>	40.5	44.6	35.1	34.6

<sup>a</sup> Value low. Good accuracy not attained on samples of such high hydroxyl content by method employed.

<sup>b</sup> Calculated acetyl value high because based on inaccurate hydroxyl value.

$$LU_1 = \pm \frac{3\sigma_{10}}{0.923}$$

When this test was applied to a low-acetyl cellulose acetate, a limit of uncertainty of  $\pm 0.13\%$  acetyl was found. In the case of acetone-soluble cellulose acetate this range is  $\pm 0.10\%$  acetyl, as compared to  $\pm 0.16\%$  acetyl when this test was applied to the same sample analyzed by the Eberstadt method. The solution method is thus a little better than the Eberstadt method. The precision attainable on the cellulose acetate butyrate I is within  $\pm 0.16\%$  apparent acetyl. The corresponding range for cellulose acetate butyrate II is  $\pm 0.21\%$  apparent acetyl. Both the precision and accuracy have been found to be a little better than obtained by the alcoholic alkali method.

The limit of uncertainty under routine conditions ( $LU_2$ ) is evaluated from similar data collected over a period of a year, and will probably be found to be somewhat larger—i.e., poorer precision.

#### TEST OF ACCURACY

It is difficult to establish the accuracy of an analytical method for cellulose derivatives because it is practically impossible to prepare a sample of known composition. A practical measure of accuracy was obtained by analyzing the four samples used in the above tests and a sample of cellulose triacetate for their free hydroxyl contents by the acetic anhydride and pyridine method (6), and the cellulose acetate butyrates for their molar ratios of acetyl and butyryl and their acetyl and butyryl contents in weight per cent (7). Acetyl values for the cellulose acetates were calculated from the observed free hydroxyl contents assuming 3 replaceable hydroxyls per glucose unit of cellulose. Apparent acetyl values for the acetate butyrates were calculated from the observed free hydroxyl contents and molar ratios of acetyl and butyryl, making the same assumption. When acetyl or apparent acetyl values, calculated in this way, agree with the observed values, a measure is obtained of the combined accuracies of all the methods involved.

Table IV shows the analytical data obtained on five samples, the number of esterified and free hydroxyls per glucose unit, and their totals. The accuracy attained in these analyses is shown by the agreement between these totals and 3 hydroxyls per glucose unit and by the agreement between the observed and calculated acetyl values.

In the case of the low-acetyl cellulose acetate the acetyl calculated does not agree well with the observed acetyl. The free hydroxyl value, however, is probably low because high accuracy of the free hydroxyl determination is not to be expected for samples having more than about 1.5 free hydroxyls. The procedure (6) does not provide drastic enough conditions to produce complete acetylation of cellulose esters having such high free hydroxyl contents. The accuracy of the analysis of this sample is therefore not established. In the other cases the observed acetyl or apparent acetyl and the calculated values agree practically

within the precision limits attainable by these methods, and the accuracy probably lies within these same limits.

#### SUMMARY

A method for the analysis of total acyl in cellulose esters of organic acids is presented in which the sample is saponified in solution. This method overcomes some of the difficulties encountered in the commonly used heterogeneous saponification methods in that it is independent of the condition of the samples, can be run in a shorter elapsed time, and is a little more accurate. It does, however, involve somewhat more complicated manipulation.

This method has been applied to solvent-soluble cellulose acetates (containing from 15 to 44.4% acetyl), cellulose acetate propionates and acetate butyrates, and cellulose propionates and butyr-

ates up to and including tributyrates. These cellulose esters have been divided into four groups based on composition and solubility. Variations are recommended for each group such that the sample is dissolved in a suitable solvent, and alkali and water are added alternately to maintain a solvent system until saponification is nearly complete. The regenerated cellulose is then in a finely divided form and does not interfere with the completion of the reaction or the back-titration.

The effects of time and temperature of saponification and time and alkali concentration were studied and the optimum conditions established. Usually 0.5-gram samples are saponified with 30 ml. of 0.5 *N* alkali with a total of 120 ml. of added solvent, allowing 6 to 16 hours for saponification at room temperature (20° to 35° C.). The reaction is complete in 6 hours or less in enough cases, particularly the cellulose acetates, so that a fairly accurate value could be obtained in one working day, if required.

Precision studies were made on samples typical of each of these groups, and it was found that precision limits of from  $\pm 0.10\%$  acetyl for cellulose acetates to  $\pm 0.20\%$  apparent acetyl for cellulose acetate butyrates may be attained when working under carefully controlled conditions. These ranges are the limits of uncertainty within which practically all carefully made determinations should fall; however, duplicate values usually agree closer than this, as shown by data reported.

A measure of the accuracy of the methods has been obtained by analyzing samples completely for acetyl, acyl groups in the case of cellulose mixed esters, and free hydroxyl. The observed acetyl or apparent acetyl results were compared with values calculated by difference from observed free hydroxyl contents and molar ratios of the acyl groups present in mixed esters, assuming exactly 3 hydroxyls per glucose unit of cellulose.

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# Determination of Vanillin and Coumarin in Flavoring Extracts

## Ultraviolet Absorption Method

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A method for the analysis of genuine and imitation vanilla extracts involves a dilution, precipitation with lead acetate, extraction with ethyl ether, and dilution of the ether extract to a large volume with water. The quantity of total vanillin and coumarin in the solution is estimated from the absorption at 2875 Å. and the quantity of each from a simple equation involving values at 2313 Å. The method is rapid and convenient and the results are of good reproducibility. Tests with mixtures of known composition indicate good accuracy. A slightly modified and simpler procedure not employing ether extraction is employed for pure vanilla extracts.

ONE of the early methods for determination of vanillin and coumarin in flavoring extracts was that proposed by Hess and Prescott (7), a modification of which is in general use today (11). This method is based on the difference in the chemical structure of vanillin and coumarin. Vanillin is separated from coumarin through the addition of aqueous ammonia to an ether extract of the vanilla extract. The vanillin forms a water-soluble aldehyde-ammonia while the coumarin remains unaffected in the ether layer. Upon acidification and extraction of the aqueous layer with ether, the vanillin is recovered in the ether layer. By evaporating the ether solutions, the amounts of vanillin and coumarin can be determined. However, the residues are usually contaminated and need further purification, thus reducing the convenience and accuracy of the method.

Several colorimetric methods for the determination of vanillin (2, 3, 6, 10) and coumarin (4, 8, 9) involve chemical reactions in which substances showing absorption in the visible spectral range are produced. However, both vanillin and coumarin show pronounced absorption in the ultraviolet region of the spectrum. Vanillin exhibits a maximum at a wave length where coumarin shows only slight absorption. It was the purpose of this work to discover if the general principle in spectrophotometry used by Englis and Skoog (5) for determination of sulfanilamide and sulfathiazole in mixtures could be similarly applied to determination of vanillin and coumarin in commercial flavoring extracts.

### EQUIPMENT

Some absorption data were obtained by means of a Bausch & Lomb medium quartz spectrograph supplemented with a Leeds & Northrup recording microphotometer. Other measurements were obtained with a Beckman photoelectric spectrophotometer.

The spectrograph was operated with a slit width of 0.07 mm. A Wood's type of hydrogen discharge tube served as a source of illumination. The hydrogen tube was placed with the exit window at a distance of 8 cm. from the slit. A cell of 1-cm. length with quartz windows was used to hold the liquids during their examination. Separate exposures of the solvents and the samples were taken for a period of 1.5 minutes each. The spectra were recorded on Eastman Polychrome plates, each of which was calibrated by making a series of separate exposures in which the time interval was varied in a regular manner: 2, 4, 8, 16, 32, and 64 seconds. The plates were developed for 5.5 minutes in Eastman x-ray developer at 18° C., then fixed, washed, and dried. After drying, the densities of the spectrograms at selected wavelength intervals were determined with a Leeds & Northrup recording microphotometer. A family of plate calibration curves for the selected wave lengths was then constructed, by reference to the appropriate curve the relative intensity values for the pure solvent and sample were found, and from these the extinction value for the solution was calculated.

The Beckman instrument was operated with a slit width of 1.0 to 1.1 mm. in the range 2250 to 2500 Å. and of 0.5 to 0.6 mm. in the range 2500 to 3000 Å. The extinction values were read directly.

### ABSORPTION CHARACTERISTICS

In establishing the absorption curves of vanillin and coumarin, solutions were prepared in concentrations of 10 mg. of each per liter in water containing about 10% alcohol. The  $E$  values are expressed for a cell length of 1 cm. The curves are shown in Figure 1; the greatest difference in absorption occurs at 2313 Å. At wave length 2875 Å. the extinction value for an equal weight of either constituent is the same. Thus the total concentrations for a mixture of the constituents can be found by determining the extinction at 2875 Å. and the amount of each individual constituent can be obtained by use of a simple equation from the extinction value at 2313 Å. The equation to be used is:

$$xE_c + (t - x)E_v = E_m$$

In the equation

$x$  = concentration of coumarin in mg. per liter

$t - x$  = concentration of vanillin in mg. per liter

$t$  = total concentration of both constituents as found by preliminary observation of  $E$  value at 2875 Å.

$E_c$  = extinction value for 1 mg. of coumarin per liter at 2313 Å.

$E_v$  = extinction value for 1 mg. of vanillin per liter at 2313 Å.

$E_m$  = extinction value observed for mixture at 2313 Å.

Subsequent testing indicated that the  $E$  values at the two wave lengths selected showed no significant change when water containing an amount of ether equivalent to that present under the conditions of analysis of later experiments was employed as a solvent.

As indicated in Figure 2, the solutions and the mixture follow the Beer-Lambert law.

### ANALYSIS OF PURE VANILLA EXTRACTS

The first portion of the experimental work was concerned with examination of samples containing only one constituent: pure vanilla extracts containing 37% alcohol. The first of these was analyzed by the official Hess-Prescott method. Percentages

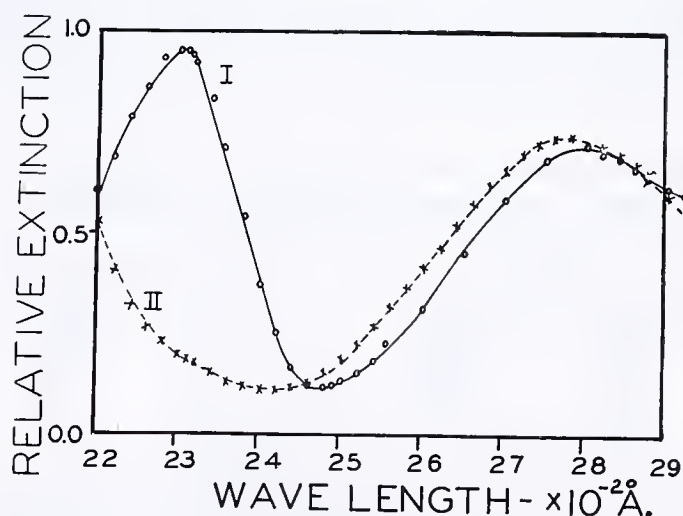


Figure 1. Absorption Curves

I. 10 mg. of vanillin per liter  
II. 10 mg. of coumarin per liter



of unpurified vanillin indicated for duplicate samples were 0.21 and 0.20%.

The object of the first experiment was to learn if vanillin could be determined on a very small sample without removing the alcohol, by precipitating the resins with lead, diluting to a larger volume, filtering, and determining the absorption of the filtrate.

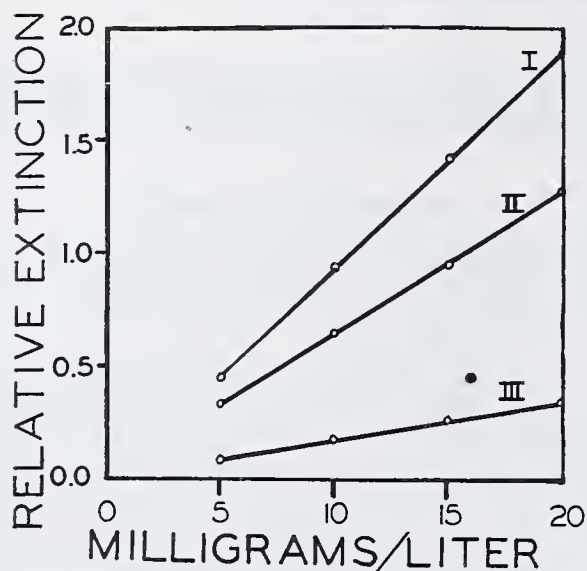


Figure 2. Absorption Values

- I. Vanillin at 2313 Å.
- II. Vanillin and coumarin at 2875 Å.
- III. Coumarin at 2313 Å.

**SPECIAL PROCEDURE FOR GENUINE EXTRACTS. NO ETHER EXTRACTION.** Five milliliters of the pure vanilla extract were measured into a 1-liter volumetric flask, 10 ml. of lead acetate (80 grams per liter) were added, and the mixture was allowed to stand for 30 minutes. At the end of this period the mixture was diluted to volume with distilled water and filtered, and the filtrate examined with the spectrophotometer. In subsequent experiments the excess lead acetate showed some absorption in the selected range. To remove the lead 20 ml. of the filtrate were treated with approximately 0.5 gram of solid sodium dihydrogen phosphate monohydrate and filtered before the absorption was determined. From the observed  $E$  values at wave length 2313 Å. the quantity of vanillin in 100 ml. of extract was calculated by reference to the  $E$  values for solutions of known concentration at this wave length. The results of the tests are given in column one of Table I.

An experiment was also carried out to determine if the method could give complete recovery of vanillin and satisfactory results if coumarin were present also.

The analyses, after addition of known amounts of pure vanillin and coumarin to the pure vanilla extract, showed recoveries of 98 to 100% and demonstrated that the principle of the analysis of the mixtures was satisfactory.

Subsequent experiments suggested that traces of other con-

stituents may have increased the absorption at the selected wave lengths and the analysis of sample I was repeated later by an alternate procedure involving an extraction with ether.

**GENERAL PROCEDURE FOR VANILLA EXTRACTS.** A 20-ml. portion of the genuine extract was measured into a 50-ml. volumetric flask and diluted to volume with distilled water. The mixture was then transferred to a 50-ml. glass-stoppered Erlenmeyer flask, 1 gram of crystalline lead acetate was added, and the mixture was shaken thoroughly and allowed to stand for 1 hour. After filtration, a 10-ml. portion of the filtrate was placed in a small separatory funnel, 10 ml. of ethyl ether (previously washed with water) were added, and the mixture was shaken. The aqueous layer was carefully withdrawn into another separatory funnel and again treated with ether. The procedure was carried out four times using successively 10-, 8-, 5-, and 5-ml. portions of the ether. After each separation the ether portion was washed into a 1000-ml. volumetric flask by means of a stream of distilled water and the funnel completely washed out. When all the ether extractions had been transferred to the volumetric flask, the mixture was diluted to volume and the ultraviolet absorption of the solution was determined. At this dilution ether is completely soluble and the solvent mixture has essentially the same absorption as water. However, a similar solution of pure water and ether is recommended for establishing the  $I_0$  value of the solvent. The ultraviolet absorption was determined at 2313 Å. as before (Table I).

The values obtained for sample I by the procedure employing the Beckman instrument are slightly lower and of somewhat better reproducibility than those obtained by the first procedure with the spectrograph. Repeating the second procedure with a new sample of similar nature and determining absorption with both types of instruments proved satisfactory, although perfect agreement was not obtained.

Table II. Analysis of Imitation Vanilla Extracts\*

(Caramel, 0.200 gram of vanillin, and 0.200 gram of coumarin per 100 ml. Ether extraction used to remove vanillin and coumarin)

Vanillin	Coumarin	Total
Gram per 100 ml.		
Spectrographic Method		
0.210	0.188	0.398
0.192	0.204	0.396
0.198	0.198	0.396
0.198	0.198	0.396
0.198	0.198	0.396
0.198	0.198	0.396
0.188	0.228	0.416
0.198	0.206	0.404
0.198	0.206	0.396
0.198	0.198	0.396
Photoelectric Spectrophotometric Method		
0.200	0.200	0.400
0.200	0.200	0.400

\* Total vanillin and coumarin found by ultraviolet absorption at 2875 Å. and proportion of each by absorption at 2313 Å.

Table I. Determination of Vanillin in Pure Vanilla Extract

(Grams of vanillin per 100 ml.)

Spectrographic Method		Photoelectric Spectrophotometric Method	
Sample I <sup>a</sup>	Sample II <sup>b</sup>	Sample I <sup>b</sup>	Sample II <sup>b</sup>
0.211	0.191	0.210	0.195
0.211	0.189	0.210	0.198
0.223	0.195	0.210	0.194
0.223	0.188	0.209	0.193
0.223	Av. 0.191	0.209	Av. 0.195
0.223		0.218	
0.224		0.218	
0.236		0.214	
Av. 0.227		Av. 0.212	

<sup>a</sup> Analyzed by special procedure. Extract clarified with lead acetate. No ether extraction used.

<sup>b</sup> Analyzed by general procedure. Extract clarified with lead acetate. Filtrate extracted with ethyl ether.

The results, after the extraction procedure, agree with the quantity indicated by the Hess-Prescott method without any purification of the residue. Perhaps there is a slight loss of vanillin in the Hess-Prescott method during removal of the alcohol and in the first drying of the sample, after evaporation of the ether. This loss may be compensated by the small amount of impurity, so that the values indicated without purification may be nearer correct.

#### ANALYSES OF IMITATION VANILLA EXTRACTS

An attempt was first made to find a clarifying agent which would remove the color but neither of the primary constituents sought. To simulate the color material in imitation vanilla extracts, samples of caramel were prepared by heating glucose (1). These were added in appropriate quantities to solutions of vanillin and coumarin, and decolorization was attempted. None of the agents tried was found satisfactory; impurities absorbing in the ultraviolet still remained or some of the constituents sought were removed. It was imperative to use an ether extraction for the separation of the vanillin and coumarin from other compo-



Table III. Analysis of Commercial Imitation Vanilla Extracts

(Ether extraction used to remove vanillin and coumarin)

Sample I			Sample II		
Vanillin	Coumarin	Total	Vanillin	Coumarin	Total
Gram per 100 ml.			Gram per 100 ml.		
Spectrographic Method			Photoelectric Spectrophotometric Method		
0.098	0.138	0.236	0.117	0.110	0.227
0.094	0.146	0.240	0.117	0.110	0.227
0.092	0.148	0.240	0.117	0.111	0.228
0.094	0.142	0.236	0.117	0.112	0.229
0.092	0.144	0.236	0.117	0.110	0.227
0.090	0.146	0.236	...	...	...
0.094	0.136	0.230	...	...	...
0.094	0.144	0.238	...	...	...
Hess-Prescott Gravimetric Method			Spectrographic Method		
0.098	0.14	0.231	0.113	0.117	0.230
0.094	0.114	0.208	0.117	0.116	0.233

Table IV. Analysis of Mixture of Genuine and Imitation Vanilla Extract by Ultraviolet Absorption Method

Sample I			Sample II		
Vanillin	Coumarin	Total	Vanillin	Coumarin	Total
Gram per 100 ml.			Gram per 100 ml.		
0.168	0.053	0.221	0.153	0.055	0.208
0.168	0.053	0.221	0.155	0.053	0.208
0.163	0.055	0.218	0.153	0.055	0.208
0.163	0.055	0.218	0.153	0.053	0.209
0.163	0.055	0.218	Av. 0.155	0.054	0.208
0.163	0.055	0.218			
Av. 0.165	0.054	0.219			
Calculated Quantities Present on Basis of Previous Analysis of Component Extracts					
0.165	0.055	0.220	0.156	0.055	0.211

nents of the imitation extracts before the spectrophotometric analysis could be carried out.

The laboratory samples of imitation vanilla extract were prepared by dissolving 0.2000 gram each of vanillin and coumarin in 10 ml. of alcohol in a 100-ml. flask, adding enough caramel to give a proper color value, and diluting to volume.

Since no resins were present, no lead clarification was carried out. A 5-ml. portion of the solution was extracted with ethyl ether, following the general procedure previously described, and the spectrophotometric evaluation made at 2313 and 2875 Å.

The quantities of vanillin and coumarin were calculated (Table II).

The results, given in Table II, show very good agreement for total vanillin and coumarin with the 0.400-gram total known to be present. Reproducibility of results is slightly less satisfactory for the individual constituents, but still very good.

The next experiment was examination of a commercial imitation extract represented as containing vanillin, coumarin, sugar, artificial color, and 2% alcohol, by the method applied to the laboratory sample (Table III). Reproducibility of results is very good for the total of the flavoring constituents and only slightly less satisfactory for the individual materials.

Duplicate analyses by the Hess-Prescott method were in good agreement with the ultraviolet absorption method.

#### BLENDS OF GENUINE AND IMITATION VANILLA EXTRACTS

It is very desirable that any method proposed for vanilla extracts be applicable to a genuine or imitation product or a blend of the two. Accordingly, the general procedure for analysis of pure vanilla extract was applied to a blend of genuine samples and commercial imitation samples already analyzed. The two were mixed in equal proportion by volume and a 20-ml. portion of the mixture was analyzed (Table IV).

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## Colorimetric Determination of Chromium in Steel

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Chromium is determined in steel by a method based on the fact that ferric perchlorate, which is itself colorless, intensifies the color of the dichromate ion. The method is not subject to interference by iron or usual alloying constituents.

IN PRACTICALLY all the colorimetric methods that have been employed, chromium in steel is determined after it has been separated from iron. The determination is then made by measuring the intensity of the chromate or dichromate color or the color produced with dichromate and a suitable organic reagent.

When large amounts of steel are taken for analysis, iron is first separated from chromium by extraction with ether. Small amounts of iron are precipitated and chromium is simultaneously oxidized and dissolved by the use of an alkaline peroxide solution. Yoe (2) describes the use of disodium-1,8-dihydroxynaphthalene-3,6-disulfonate (Koenig's reagent), diphenylcarbazine, and diphenylsemicarbazide for the colorimetric analysis of chromium. These reagents are very sensitive to small amounts of chromium but are subject to interference by iron and other alloying constituents that may be present in certain steels, thus making chemi-

cal separations necessary. However, Mal'tsev and Temirenko (1), using diphenylcarbazine, take a small sample weight and determine up to 0.1% chromium in steel without previous separation. Organic reagents have been employed in cases where the chromium content of steel is very low, as the color produced by small amounts of chromate or dichromate ion alone is too weak to allow accurate results.

This paper describes a method for the colorimetric determination of chromium in steel, which is not subject to interference by iron or alloying constituents usually present. The method is suitable for steel containing between a few thousandths and 1% chromium and is rapid, as chemical separations are not used. The basis of the method lies in the fact that ferric perchlorate, which is itself colorless, intensifies the color of the dichromate ion.

#### EXPERIMENTAL

Solutions were prepared containing known amounts of chromium and iron, using a standard dichromate solution and National Bureau of Standards sample 22b. These samples were dissolved and boiled with perchloric acid to oxidize chromium. After being cooled and diluted to a definite volume they were transferred to an absorption tube and a colorimeter reading was taken. The solutions were then reduced with a small crystal

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Table I. Effect of Iron on Intensity of Dichromate Color

Chromium, Mg.	1.000 Gram of Iron			0.500 Gram of Iron			No Iron		
	Oxi- dized solution	Re- duced solution	Dif- ference	Oxi- dized solution	Re- duced solution	Dif- ference	Oxi- dized solution	Re- duced solution	Dif- ference
0.070	13	0	13	...	..	...	...	..	...
0.240	42	2	40	...	..	...	...	..	...
0.570	91	3	88	...	..	...	...	..	...
0.870	132	4	128	...	..	...	...	..	...
0.950	150	5	145	78	1	77	37	0	37
1.15	170	6	164	...	..	...	...	..	...
1.85	272	7	265	152	4	148	72	0	72
2.30	332	10	322	188	5	183	90	1	89
2.75	382	11	371	222	7	215	109	2	107
3.65	464	13	451	289	8	281	145	3	142
4.55	...	..	...	354	8	346	182	5	177
5.55	...	..	...	427	11	416	222	7	215

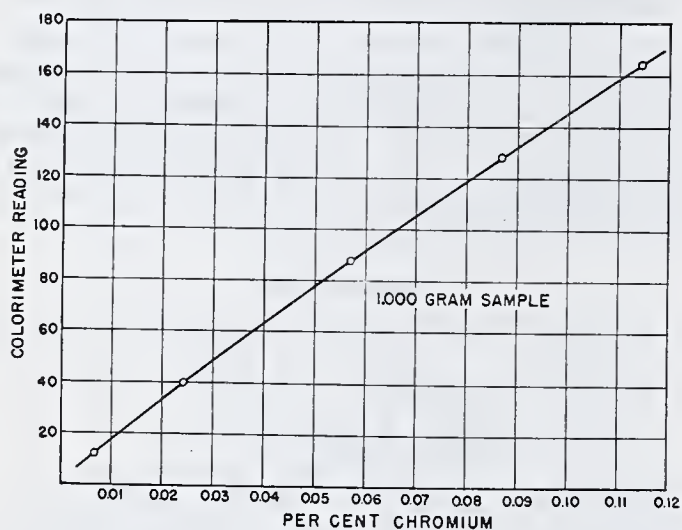


Figure 1

of ferrous ammonium sulfate and a second reading was taken. The difference between these two readings represents the colorimeter reading due to the dichromate.

Results in Table I show that ferric perchlorate itself is colorless but increases the color intensity of the dichromate.

It was found from subsequent experiments that a 1-gram sample was most suitable when the chromium content of the steel was between a few thousandths and 0.1%. When the percentage of chromium was between 0.1 and 1%, a 0.5-gram sample was taken for analysis.

Accordingly, solutions were prepared using a steel of known chromium content and a standard solution of potassium dichromate. Readings were taken on these solutions after carrying

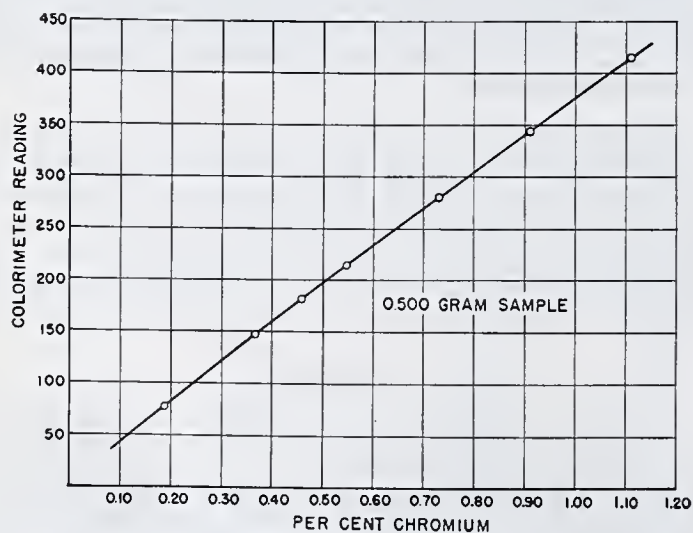


Figure 2

them through the procedure described below. Graphs (Figures 1 and 2) were drawn from the results obtained. In each case the line is a slight curve.

In order to determine the accuracy of the method, various samples of Bureau of Standard steels were analyzed. Artificial standards were also prepared by adding nickel, copper, cobalt, molybdenum, aluminum, titanium, and vanadium to weighed mixtures of Bureau of Standard steels and analyzing the resulting mixtures (Table II).

#### PROCEDURE

**PREPARATION OF SOLUTION.** Transfer 1.000 gram of sample, for steel containing up to 0.1% chromium, to a 125-ml. Phillips beaker and dissolve in 10 ml. of dilute nitric acid (1 to 1) and 20 ml. of perchloric acid (70 to 72%). For steel containing between 0.1 and 1% of chromium take a 0.500-gram sample and dissolve in 10 ml. of nitric acid (1 to 1) and 15 ml. of perchloric acid (70 to 72%). Evaporate to dense fumes of perchloric acid and boil gently for 5 minutes to oxidize chromium (boiling for 8 minutes does no harm). Cool the beaker and contents rapidly in tap water. Dissolve soluble salts with 20 ml. of water and transfer the solution to a 50-ml. glass-stoppered volumetric flask. Cool the solution to room temperature and dilute to 50 ml.

**TAKING THE COLORIMETER READING.** Transfer a portion of the solution to the absorption tube and reduce with a small crystal (about 10 to 20 mg.) of ferrous ammonium sulfate. Adjust the colorimeter so that the reading on this solution is zero. Discard the solution in the absorption tube, refill with the oxidized solution, and take a second reading. This reading is a measure of the color due to the dichromate. In the absence of elements which form highly colored ions, such as copper, nickel, cobalt, etc., it is not necessary to reset the colorimeter at zero.

Table II. Determination of Chromium in Bureau of Standards Samples

Composition Gram	Alloying Element Added %	Chromium Present %	Chromium Colorimetrically %
1.000 8d	None	0.007	0.007
1.000 11d	None	0.008	0.008
1.000 13d	None	0.023	0.023
1.000 15c	None	0.055	0.055
0.500 30c	None	0.977	0.98
0.500 72	None	0.911	0.91
0.500 72a	None	0.655	0.66
0.500 100	None	0.180	0.19
0.300 20d	None	0.087	0.087
0.700 22b	None	0.115	0.115
0.400 20d	None	0.022	0.022
0.600 22b	None	0.095	0.099
0.970 13d	3 Cu	0.175	0.187
0.500 100	3 Cu	0.883	0.90
0.470 11d	3 Ni	0.022	0.020
0.485 100	3 Ni	0.095	0.096
0.485 72	3 Ni	0.175	0.18
0.970 13d	3 Ni	0.883	0.87
0.500 11d	3 Ni	0.095	0.096
0.500 68 <sup>a</sup>	1.38 Mo	0.108	0.092
0.400 72	0.67 Mo	0.764	0.75
0.100 106	0.1 Al	0.137	0.137
0.250 106	0.5 Al	0.65	0.64
0.250 11d	1 Co	0.023	0.021
0.990 13d	1 Co	0.092	0.095
0.500 100	1 Co	0.178	0.186
0.490 22b	1 Co	0.902	0.90
0.495 72	1 V	0.902	0.88
0.495 72	1.5 Ti	0.115	0.102
1.000 HJN <sup>b</sup>	0.75 Ti	0.51	0.49
0.250 HJN <sup>b</sup>	0.50 Ti	0.646	0.63
0.25 72			
0.167 HJN <sup>b</sup>			
0.333 72			

<sup>a</sup> Authors' alloy, contains 2.76% molybdenum and 0.208% chromium.  
<sup>b</sup> Authors' alloy, contains 1.50% titanium and 0.115% chromium.



All subsequent colorimeter measurements are made by taking a reading on an oxidized solution, reducing the solution in the absorption tube with a small crystal of ferrous ammonium sulfate, and taking a second reading. The difference between the first and second readings then represents the color due to the dichromate present. If appreciable amounts of highly colored ions are present, the colorimeter is adjusted to read zero on the reduced solution before a reading is taken on the oxidized sample.

#### DESCRIPTION OF COLORIMETER

A Klett-Summerson photoelectric colorimeter was used. This colorimeter has a logarithmic scale, and when Beer's law applies, the scale readings are proportional to the concentration of colored ion. Measurements were made in an absorption tube of 12.5-mm. inside diameter, with a color filter transmitting between 410 and 480 millimicrons.

#### DISCUSSION OF RESULTS

Reference to Table II shows that duplicate determinations agree well and the results obtained compare favorably with the Bureau of Standard certificate values. Moderate amounts of the ordinary alloying constituents do not affect the accuracy of the method. Since the color intensity of dichromate is depen-

dent upon the concentration of ferric perchlorate, it is evident that large amounts of alloying constituents would produce an appreciable error in this method. In the case of silicon, for example, experience has shown that amounts in excess of 1% cause a perceptible error if the normal graph of per cent *vs.* colorimeter reading is used. However, supplementary graphs for the determination of chromium in high-silicon iron or steel may be prepared, by using samples which contain known chromium and known, similar, high-silicon percentages. An additional precaution deserves mention: it is necessary to allow the silica present in the colorimeter tube to settle for 1 or 2 minutes before taking a reading.

This method is applicable to the great majority of steels without any modification whatever. Inasmuch as iron is not separated from chromium, the analysis is more rapid than most methods for the colorimetric determination of chromium in steel.

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## Quantitative Separation of Alcohol and Ester Forms of Vitamin A

### By Solvent Extraction and Chromatographic Methods

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If vitamin A alcohol and vitamin A palmitate are distributed between equal volumes of 95% aqueous methanol and petroleum ether, 27% of the alcohol form and 98% of the ester form remain in the petroleum ether phase. A simple formula allows calculation of the percentage of either form of vitamin A if an unknown mixture is distributed between the solvents. Vitamin A alcohol can be separated quantitatively from its esters by filtration of a solution in ethylene dichloride through chromatographic columns of activated alumina. A procedure for carrying out this analytical separation is described.

IT HAS been claimed (4) that esterified vitamin A in its natural state in fish liver oils has a higher biological potency than equivalent amounts of vitamin A alcohol in the unsaponifiable fractions of these oils. Investigation of these claims necessitated the development of a fast and simple method for the analytical separation of vitamin A alcohol from its esters. Molecular distillation of oils has been used (6), but the equipment is not so simple in operation as is desirable for an analytical method. (A method for the assay of both forms by fluorophotometry was published after the completion of this manuscript, 8.)

The analytical separation by solvent fractionation was studied by Gillam (5) who reported the distribution of vitamin A (alcohol) between light petroleum ether and aqueous methanol. Claussen (3) separated the alcohol and ester forms by distribution between hexane and 81.9% aqueous ethanol. A search of the literature did not reveal data concerning the advantages of different alcohols for the separation or details of the analytical procedure.

The distribution of crystalline vitamin A alcohol and crystalline vitamin A palmitate was determined by shaking a solution of about 0.40 mg. of vitamin A alcohol or 0.70 mg. of vitamin A ester in 50 ml. of petroleum ether (Skellysolve B) with an equal volume of an aqueous aliphatic alcohol. The change in volume was noted and the percentage distribution was calculated from deter-

minations of vitamin A in the petroleum ether phase by measuring the ultraviolet absorption at 328 m $\mu$ . The results are summarized in Figure 1.

The percentage distributions agree well with those reported by Gillam (5, 70, 80, and 95% aqueous methanol), and Claussen (3, 82% aqueous ethanol), and Baxter (1, 83% aqueous ethanol). The distribution refers to the amount of vitamin A in each phase rather than to its concentration, as the volume of the phases changes owing to mutual solubility of the solvents. The volume of 50 ml. of 95% methanol increases on shaking with 50 ml. of petroleum ether to 57 ml. at 5.5°, 59 ml. at 28.5°, and 60 ml. at 36°.

The best separation for analytical purposes can be obtained with 95% (by volume) aqueous methanol, as only 27% of the al-

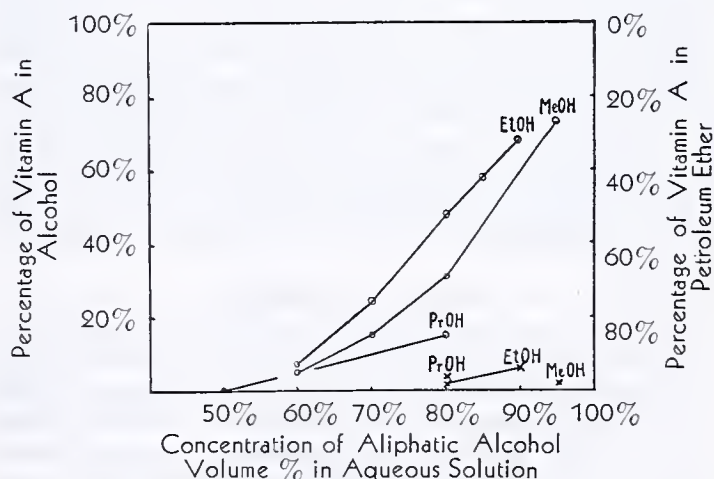


Figure 1. Distribution of Vitamin A Alcohol and Ester Forms between Aliphatic Alcohols and Petroleum Ether

O. Vitamin A alcohol  
X. Vitamin A ester



Table I. Adsorption of Vitamin A

Adsorbent	(40 I.U. per ml.)	
	Ester Adsorbed %	Alcohol Adsorbed %
From 100 ml. of ethylene dichloride on 5 grams of adsorbent		
Powdered dextrose	0	0
ZnO (activated)	0	0
Magnesol	0	17
Ca <sub>2</sub> P <sub>2</sub> O <sub>7</sub> · 4H <sub>2</sub> O	0	13
Ca(OH) <sub>2</sub>	0	33
Al <sub>2</sub> O <sub>3</sub> (activated)	0	70
On chromatographic columns, 1.1-cm. diameter, 10.5-cm. length		
Powdered dextrose	0	0
ZnO (activated)	0	26
Ca <sub>2</sub> (PO <sub>4</sub> ) <sub>2</sub>	0	58
Al <sub>2</sub> O <sub>3</sub>	0	100

Table II. Ester Content of Oils and Concentrates

Sample	Ester	
	I.U./g. (approx.)	%
Vitamin A acetate	3,500,000	100
Vitamin A palmitate	3,300,000	98
Fish liver oil (shark)	115,000	98
Vitamin A distillate	200,000	98
Halibut liver oil	130,000	94
Cod liver oil	2,500	93
Vitamin A alcohol	4,000,000	1
Fish liver oil concentrate	1,000,000	0

cohol form and as much as 98% of the ester form remain in the petroleum ether phase.

**ANALYTICAL SOLVENT SEPARATION.** Dissolve a sample of fish liver oil or concentrate containing about 0.5 to 5 mg. of vitamin A (in the following, the weight of vitamin A esters is expressed as the weight of its contents of vitamin A alcohol) in 50 ml. of petroleum ether. Shake vigorously with 50 ml. of 95% aqueous methanol in a 100-ml. glass-stoppered measuring cylinder for 1 minute, allow the phases to separate, and note the volumes. Determine the concentration of vitamin A in each phase with the antimony trichloride reaction or spectrographically after proper dilution. Calculate the milligrams of vitamin A in each phase and find the milligrams of vitamin A alcohol and ester from the following formulas:

$$a = 1.380 \times A_m - 0.028 \times A_e$$

$$e = 1.028 \times A_e - 0.380 \times A_m$$

where  $a$  represents mg. of vitamin A alcohol,  $e$  mg. of vitamin A ester,  $A_m$  mg. of vitamin A in the methanol phase, and  $A_e$  mg. of vitamin A in the petroleum ether phase.

The solvent separation cannot be applied if vitamin A is present as the acetate ester, as only 78% of the acetate remains in the petroleum ether phase. The presence of 1% of cholesterol affects the distribution considerably (5). The presence of more than 1% of the fish liver oil or concentrate tends to shift the distribution of vitamin A alcohol to the petroleum ether phase.

Alumina has been widely used for the adsorption of the alcohol form of vitamin A concentrates. During the preparation of vitamin A stearate Mead (7) removed the unchanged vitamin A alcohol by percolating a petroleum ether solution through a column of activated alumina. Swain (9) found that the alcohol form of vitamin A is much more easily adsorbed on alumina than its esters. This chromatographic method was promising for the quantitative analytical separation, particularly as a similar method for the quantitative separation of cholesterol from its esters has been reported (10).

Experiments with different solvents showed that quantitative separation for the widest range in concentration could be obtained with ethylene dichloride. Solvents of greater eluant power allowed some vitamin A alcohol to pass a column of alumina, whereas petroleum ether and cyclohexane allowed some vitamin A ester to remain in the column. Adsorbents were tested by shaking a sample of 5 grams with 100 ml. of solutions of vitamin A alcohol or palmitate (about 1 mg. in 100 ml. of ethylene dichloride). (The author is obliged to J. G. Baxter for a sample of pure vitamin A palmitate.) The results are shown in

Table I, which also shows the adsorption on chromatographic columns, using the first 5 ml. of filtrate for analysis.

The degree of activation of the alumina is of the greatest importance. Alumina which has been too highly activated will destroy part of the vitamin A ester during passage. The destruction can easily be recognized by the shape of the ultraviolet absorption curve. It is not always evident in the antimony trichloride reaction, as some decomposition products will still develop a blue color with the reagent. Columns of alumina which were well suited to the quantitative separation did not adsorb azobenzene from a 1 to 4 mixture of benzene and petroleum ether. They permitted the separation of 4-methoxyazobenzene and benzene-azo- $\beta$ -naphthol from the same solvent into two distinct bands on the column. Benzene-azo- $\beta$ -naphthol and aminoazobenzene-azo- $\beta$ -naphthol were so strongly adsorbed that they could not be separated on the column. This indicates a degree of activation of group 2 in Brockmann's standardization of alumina (2).

**ANALYTICAL SEPARATION BY CHROMATOGRAPHY.** Introduce activated alumina, Alorco Grade A, <80-mesh, into a 50-ml. stopcock buret and secure a column of 15 ml. (about 15 grams) in the lower part with plugs of Pyrex glass wool. Activate the column at from 110° to 120° C. for 1.5 hours in a stream of nitrogen. Draw about 30 ml. of ethylene dichloride with gentle suction through the column in order to avoid any deleterious effect by the heat of wetting, allowing the level of the liquid to stay just above the column. Add exactly 2 ml. of a solution of a sample of fish liver oil or concentrate (containing about 5 mg. of vitamin A in 10 ml. of ethylene dichloride) and pass through the column. Follow immediately with 40 to 50 ml. of ethylene dichloride, never allowing the column to become dry. Transfer the filtrate to a 100-ml. measuring flask and fill up to volume. Assay this solution and the original solution (after diluting 1 to 50) with the antimony trichloride reaction. The concentration of vitamin A found in the filtrate is due to the ester form alone. The concentration of the alcohol form is found by subtraction from the total concentration.

With the above concentrations of vitamin A, the alcohol form will not form a colored band on the column, so that its progress cannot be observed directly. If the column is allowed to stand for one day, a yellow-orange band, due to vitamin A decomposition products, will become visible about 2 to 3 cm. below the top of the column. The ultraviolet absorption at 328  $m\mu$  can be used for the estimation of vitamin A in the filtrate, but allowance must be made for the fact that the absorption of vitamin A in ethylene dichloride is about 13% less than in isopropanol.

Crystalline vitamin A alcohol does not pass the column under the above experimental conditions and no increase of the concentration in the filtrate is observed after the addition of the alcohol to fish liver oils. Crystalline vitamin A palmitate and acetate pass the column without changing concentration and can be recovered in the filtrate after addition to fish liver oils. Results obtained by this method are reproducible within 2% and agree within these limits with results obtained by the solvent extraction procedure. Table II shows the ester content of some representative samples of oils and concentrates.

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# Estimation of Tung Oil as an Adulterant

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**T**UNG oil is sometimes adulterated with soybean, sesame, peanut, rapeseed, stillingia, and other oils, which are much cheaper than tung oil during normal times. Since the present Sino-Japanese War, the situation has been reversed. As the facilities for exporting tung oil from China abroad have been greatly reduced and almost cut off, its price in China has become far lower than the other kinds of ordinarily available vegetable oils. In spite of the fact that large quantities of tung oil have been consumed by the new-born tung oil cracking industries in the manufacture of gasoline and fuel oil substitutes, its price, relative to other oils, still remains low. As a consequence it is not an uncommon practice of some dishonest dealers to use tung oil as an adulterant in other higher-priced edible oils—e.g., rapeseed, peanut, and sesame oils.

On account of its strong purgative and emetic action tung oil is not edible. The presence of a small amount of tung oil in other edible oils, even as little as 0.5%, may cause severe disturbances, if the oils are taken as food. On the other hand, when rapeseed and other oils are used industrially, especially to make lubricating oil components or substitutes, the presence of tung oil is highly objectionable because of its tendency to gelatinize. It is obvious that the detection and determination of adulterating tung oil in these oils are of considerable importance.

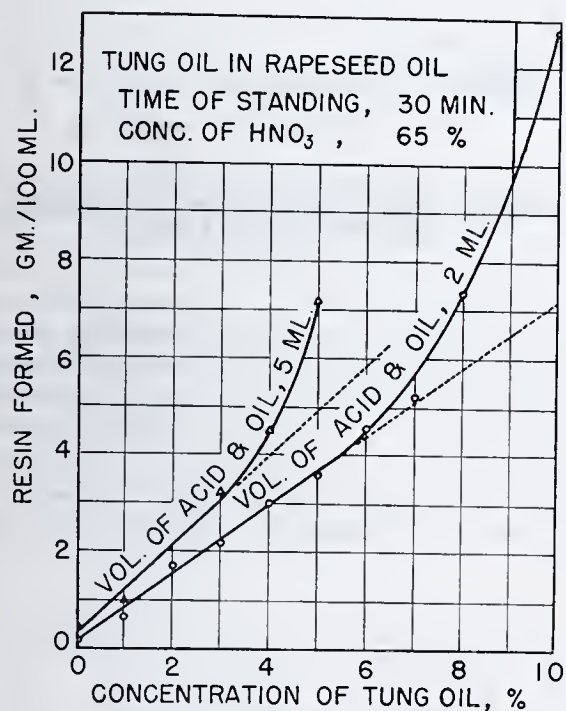


Figure 1. Resins Formed from Samples of Rapeseed Oil Adulterated with Tung Oil

Several analytical methods for testing the purity of tung oil and determining the amount of its adulterants present are available (4, 5), largely based on the gelatinization of tung oil upon heating. When the percentage of tung oil in the mixtures is small, these methods cannot be applied. Tung oil is characterized by its high refractive index and high iodine number, and an examination of these values of the oils under consideration may throw some light on the presence or absence of tung oil. However, as these values of the unadulterated oils cover a comparatively wide range, they do not give conclusive evidence.

A test based on some reaction specific only to tung oil will be much more desirable.

## COLORIMETRIC QUALITATIVE TEST

A qualitative test for tung oil has been worked out by Wan (3, 6), its procedure being essentially the following:

The testing reagent is prepared by dissolving 10 grams of antimony trichloride in 100 ml. of chloroform, 5 to 10 ml. of this reagent are poured into a test tube, and 1 drop of the oil to be examined is placed on the surface. On standing for 10 minutes to 1 hour, the formation of a dark red ring indicates the presence of tung oil.

This test is very sensitive. Although castor oil and sesame oil in this reagent after long standing may also produce a pinkish tint, the intensity of color due to the presence of tung oil is far more pronounced.

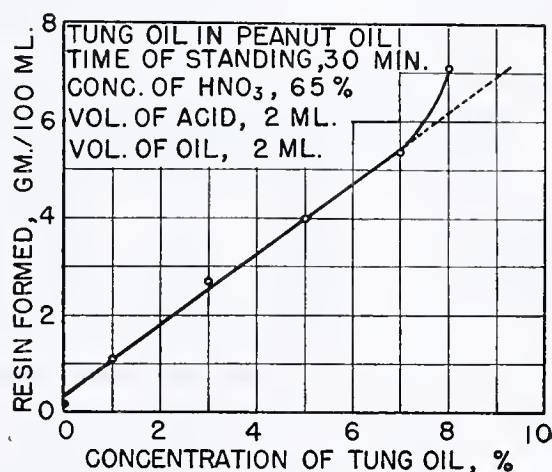


Figure 2. Resins Formed from Samples of Peanut Oil Adulterated with Tung Oil

The present authors attempted to develop this test into a quantitative colorimetric method. Samples of 0.1 to 0.2 gram of rapeseed oil containing a few per cent of tung oil were weighed out in test tubes, and 10-ml. portions of the antimony trichloride reagent were added to each. They were thoroughly shaken and let stand for 2 or 3 hours. The solutions were then filtered and immediately compared in a colorimeter. It was found that the color intensity was not directly proportional to the concentration of tung oil present. Furthermore, the reaction seemed still to go on, and precipitates continued to form even during the short time of examination in the colorimeter. This greatly interfered with the comparison of the color. Only after very long standing, say, overnight, did the filtered solution remain clear. This reaction was therefore discarded as the basis of a method for quantitative determination.

## GRAVIMETRIC METHOD

When tung oil is treated with concentrated nitric acid a solid jellylike mass is formed in a short time (1, 2). Based on this reaction the following gravimetric method was worked out.

A few milliliters of the oil sample under test are treated with concentrated nitric acid in a glass-stoppered test tube. After being thoroughly shaken, it is immersed in ice water for some time. Then the reaction product is filtered through an asbestos-matted Gooch crucible, washed thrice with petroleum naphtha (50° to 150° C.), dried at 100° C., and weighed.



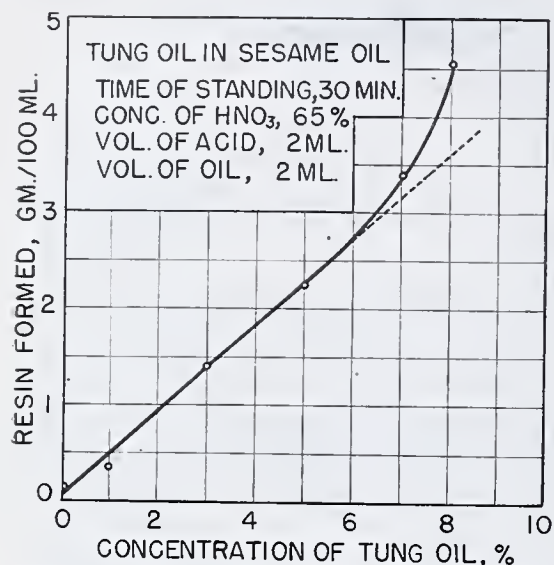


Figure 3. Resins Formed from Samples of Sesame Oil Adulterated with Tung Oil

It was found that the result was affected by the following variables: nature of the adulterated oil, concentration of nitric acid, time of standing, quantity of sample, and quantity of nitric.

Table I. Characteristics of Oils Used

	Tung Oil	Rapeseed Oil	Peanut Oil	Sesame Oil
Specific gravity (15.5° C.)	0.941	0.914	0.918	0.922
Solidifying point, ° C.	2.8	1.4720	1.4691	1.4719
Refractive index (25° C.)	1.5183	1.4720	1.4691	1.4719
Acid number	5.7	5.5	5.2	9.3
Saponification number	192.5	187.9	196.2	186.6
Iodine number (Wijs)	171.8	102.7	98.2	112.4
Titer test, ° C.	32.2	15.8	34.6	21.2
Heating test (A.S.T.M.), minutes	9.3	....	....	....

Table II. Resins Formed under Different Conditions

Expt. No.	Adulterated Oil	Concentration of Tung Oil %	Concentration of HNO <sub>3</sub> %	Volume of Oil ML.	Volume of Acid ML.	Time of Standing Min.	Resin Formed G./100 ml.
R-0	Rapeseed	Blank	65.0	2	2	30	0.15
R-1	Rapeseed	1	65.0	2	2	30	0.65
R-2	Rapeseed	2	65.0	2	2	30	1.70
R-3a	Rapeseed	3	65.0	2	2	30	2.05
R-3b	Rapeseed	3	65.0	2	2	30	2.25
R-3c	Rapeseed	3	65.0	2	2	30	2.10
R-4	Rapeseed	4	65.0	2	2	30	3.00
R-5	Rapeseed	5	65.0	2	2	30	3.60
R-6	Rapeseed	6	65.0	2	2	30	4.55
R-7	Rapeseed	7	65.0	2	2	30	5.25
R-8	Rapeseed	8	65.0	2	2	30	7.36
R-10	Rapeseed	10	65.0	2	2	30	12.75
R-11	Rapeseed	Blank	65.0	5	5	30	0.44
R-12	Rapeseed	1	65.0	5	5	30	0.99
R-13	Rapeseed	3	65.0	5	5	30	3.20
R-14	Rapeseed	4	65.0	5	5	30	4.50
R-15	Rapeseed	5	65.0	5	5	30	7.22
R-21	Rapeseed	3	65.0	2	2	60	2.53
R-22	Rapeseed	3	65.0	2	2	120	3.05
R-23	Rapeseed	5	65.0	2	2	60	5.20
R-24	Rapeseed	5	65.0	2	2	120	5.75
R-25	Rapeseed	3	65.0	2	5	30	2.30
R-26	Rapeseed	3	65.0	2	10	30	2.35
R-27	Rapeseed	3	65.0	2	10	60	2.55
R-28	Rapeseed	3	65.0	2	10	120	2.75
R-29	Rapeseed	8	65.0	1	1	30	7.70
R-30	Rapeseed	10	65.0	1	1	30	12.40
R-31	Rapeseed	10	65.0	1	2	30	14.00
R-32	Rapeseed	5	70.0	2	2	30	7.95
R-33	Rapeseed	5	60.0	2	2	30	0.20
P-0	Peanut	Blank	65.0	2	2	30	0.16
P-1	Peanut	1	65.0	2	2	30	1.10
P-3	Peanut	3	65.0	2	2	30	2.70
P-5	Peanut	5	65.0	2	2	30	4.01
P-7	Peanut	7	65.0	2	2	30	5.35
P-8	Peanut	8	65.0	2	2	30	7.10
S-0	Sesame	Blank	65.0	2	2	30	0.15
S-1	Sesame	1	65.0	2	2	30	0.35
S-3	Sesame	3	65.0	2	2	30	1.40
S-5	Sesame	5	65.0	2	2	30	2.25
S-7	Sesame	7	65.0	2	2	30	3.40
S-8	Sesame	8	65.0	2	2	30	4.55

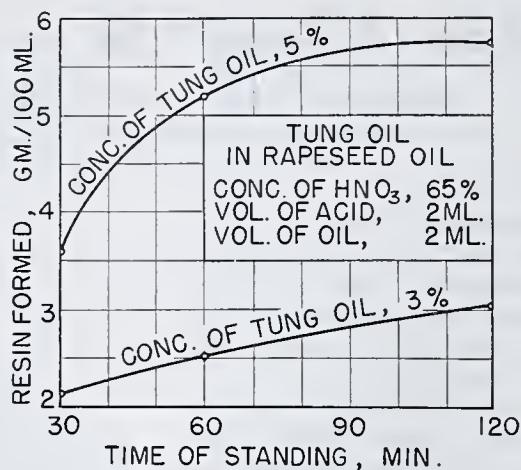


Figure 4. Effect of Time of Standing on Quantity of Resin Formed

acid. They were studied in detail and the best conditions sought.

The experimental results under various conditions are given in Table II and Figures 1 to 6. The nearly straight-line relationship between the weight of the resinous matter formed and the percentage of tung oil in the adulterated oils (Figures 1 to 3), when its concentrations are low, indicates that this method can be used as a

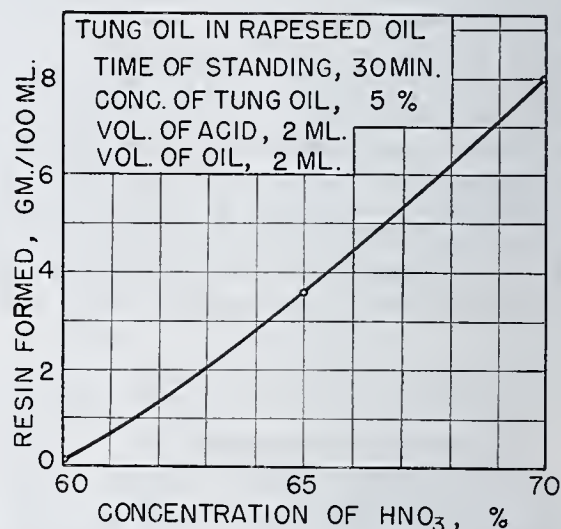


Figure 5. Effect of Concentration of Nitric Acid on Quantity of Resin Formed

quantitative measurement of the amount of tung oil present.

This method is far from ideal. The amount of resin formed per unit amount of tung oil present varies greatly with the experimental conditions. In general, it increases with longer times of standing (Figure 4) and higher concentrations of nitric acid (Figure 5), and varies slightly with the amount of nitric acid used (Figure 6 and experiments R-22, R-28, R-30, and R-31).

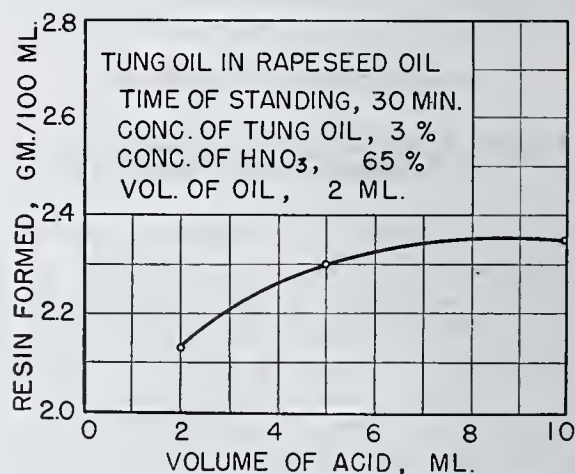


Figure 6. Effect of Volume of Nitric Acid on Quantity of Resin Formed



Among these factors the concentration of nitric acid has a tremendous effect. If the concentration is below 60%, there is hardly any resin formed. If it is above 70%, the jellylike mass is so voluminous and thick that is difficult to handle. It was found that 65% strength is about right. In the actual determinations of tung oil content, the concentration of nitric acid should obviously be accurately adjusted.

It seems that the resin is not formed solely from the tung oil. The adulterated oil is also somehow affected, probably coprecipitated, but to different degrees for different oils. The quantity of resin formed per unit quantity of tung oil present differs appreciably in rapeseed, peanut, and sesame oils. This shows that the adulterated oil also takes part in the process of resinification. With the same kind of adulterated oil, the quantity of resin formed becomes higher when the tung oil concentration or the total amount of resin goes beyond a certain limit. The results with 1-ml. and 2-ml. portions of oils and acid are fairly close to each other (experiments R-8 and R-10, R-29 and R-30). When the volume of the samples and acid used is increased to 5 ml., however, the quantity of resin formed becomes noticeably higher.

The mechanism of the reaction between tung oil and nitric acid has not been clear. Considering all the variations men-

tioned above, it appears to be complicated. For our present purpose, however, it can be utilized as a basis for quantitative estimation of tung oil as an adulterant. By weighing the resin obtained under controlled conditions and comparing it with a previously prepared curve with known samples under identical conditions, the percentage of tung oil present can be readily ascertained. The reproducibility of the experimental results is reasonably high, as evidenced by experiments R-3a, R-3b, and R-3c. If the percentage of tung oil in the adulterated oil is too high, it may be first diluted with clean oil. However, such cases would be rare. The recommended experimental conditions are 65.0% nitric acid, 2.0 ml. each of oil sample and acid, and 30-minute standing in ice water.

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## Determination of Carotene in Dehydrated Alfalfa

### A Simplified Method

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A simplified method has been developed for the determination of carotene in dehydrated alfalfa. The finely ground alfalfa is allowed to stand for 16 to 18 hours in a mixture of acetone and Skellysolve B, the extract is concentrated on a steam bath to remove the acetone, the pigments are adsorbed by drawing the mixture through a magnesia column, and the carotene is then eluted with a 4% solution of acetone in Skellysolve B. Refluxing and phasic separations are avoided and many more samples can be handled per day than by other methods in common use.

IN CONNECTION with a research project at this laboratory which involves a study of the changes occurring in the carotene content of alfalfa during dehydration and storage, a rapid as well as accurate method for estimating the carotene content of a large number of dehydrated samples became desirable. It was hoped that a procedure could be found which would not require phasic separations, since both time and equipment would be saved. A method which required little heating was also desired, in order to hold the isomerization of carotene to a minimum during the analytical procedure.

A survey of the more common methods of analysis showed no one procedure that completely met the needs. It was not possible to extract all the carotene by the method suggested by Kernohan (6) which involves allowing the sample to stand in petroleum ether. It was found, however, that the carotene could be extracted by allowing a sample of dehydrated alfalfa to stand in a mixture of Skellysolve B and acetone. Wall and Kelley (13) used these solvents in the extraction of dehydrated materials in a Soxhlet. It has been found convenient to weigh out the samples in the afternoon and allow them to stand overnight. This procedure makes it possible to determine the carotene content of a large number of samples per day. Results are re-

producible with an accuracy equal to or somewhat better than those obtained in this laboratory by other methods. Isomerization of the carotene is minimized and a saving in equipment is effected, since phasic separations may be eliminated and complicated extraction apparatus is not required.

#### PROCEDURE

**EXTRACTION OF CAROTENE.** One or 2 grams of finely ground alfalfa (preferably through a 40-mesh screen) are weighed into an Erlenmeyer flask or sample bottle and covered with 60 ml. of a mixture of 1 part acetone to 2 parts Skellysolve B. The mixture is shaken and the tightly stoppered container then set aside in the dark for 16 to 18 hours, usually overnight. The extract is filtered through a Büchner funnel and the residue thoroughly washed, by decantation, with several small portions of Skellysolve, followed by heating on a steam cone to drive off most of the remaining acetone and to concentrate the solution to a volume of approximately 40 ml. (15 minutes' heating is usually sufficient).

**SEPARATION OF CAROTENE FROM OTHER PIGMENTS.** A chromatographic separation of carotene from other pigments is made on a column of 2 parts Hyflo Super-Cel and 1 part magnesia (Micon brand No. 2641). The columns are made as described by Wall and Kelley (13) but are shorter in length (8 to 10 cm.). After adsorption of the pigments, the carotene is eluted with a 4% acetone-Skellysolve B mixture.

**ESTIMATION OF CAROTENE.** The solution of carotene is made up to volume and analyzed with a Beckman (2) spectrophotometer, for  $\beta$ -carotene and neo- $\beta$ -carotene B, using the wave lengths and absorption coefficients suggested by Beadle and Zscheile (1).

#### DISCUSSION

The procedure has been checked against two of the more common methods of analysis. The method of Peterson, Hughes, and Freeman (10) gave slightly higher carotene values in most cases, but the thoroughness of the methanol wash has considerable



Table I. Comparison of Three Methods for Determination of Carotene in Dehydrated Alfalfa

Sample No.	P.H.F. Method		Moore and Ely Extraction Column MgO		Proposed Method	
	Total carotene	$\beta$ -Carotene	Total carotene	$\beta$ -Carotene	Total carotene	$\beta$ -Carotene
	Mg./100 g.	%	Mg./100 g.	%	Mg./100 g.	%
316 <sup>a</sup>	38.2	70	38.5	89	37.6	86
	38.0	69	38.7	85	38.2	86
321	17.4	67	16.8	84	16.6	83
	16.1	73	16.1	83	16.3	82
325 <sup>a</sup>	37.8	75	37.8	86	36.5	88
	38.0	74	38.0	86	36.5	88
331	13.0	67	13.0	76	13.0	80
	12.0	67	12.5	75	12.8	78
341	20.4	67	20.3	83	20.2	84
	19.6	66	19.8	85	19.4	89
510	11.4	58	11.1	81	10.9	81
	11.2	56	11.0	84	10.6	86

<sup>a</sup> Steam-blached before drying.

Table II. Effect of Removing Noncarotenoid Pigments from Carotene Extracts Obtained by Phasic Separation

Sample No.	P.H.F. Method		P.H.F. Method Followed by Adsorption		Adsorption Procedure	
	Total carotene	$\beta$ -Carotene	Total carotene	$\beta$ -Carotene	Total carotene	$\beta$ -Carotene
	Mg./100 g.	%	Mg./100 g.	%	Mg./100 g.	%
331	13.0	67	11.9	72	12.8	78
382	16.1	57	12.6	72	11.8	76
383	16.8	53	12.4	67	12.6	70
384	23.0	67	18.9	73	16.3	72
385	9.1	57	7.6	70	8.2	83

influence on the results obtained by this method. The data in Table I were obtained with a more rigorous washing procedure and agree closely with chromatographic techniques. The apparently low percentage of  $\beta$ -carotene calculated when the Peterson, Hughes, and Freeman (10) technique of extraction was used is shown in Tables I and II. These low values are due, largely, to the presence of small amounts of noncarotenoid pigments, which cause the absorption measurements at 478 m $\mu$  to be low. It has been pointed out by several workers (3, 7, 14) that some noncarotenoid pigments remain after phasic separations. Table II shows the increase in per cent of  $\beta$ -carotene and the lower carotene values following the removal of the noncarotenoid pigments on a magnesia column.

The other method used for comparison was an extraction procedure, using a Waring Blendor and a foaming solvent mixture of alcohol and Skellysolve B similar to that of Moore and Ely (8). This was followed by adsorption and elution from a magnesia column according to the method of Wall and Kelley (13).

There is good agreement between the data obtained by the proposed method and the other two methods, as shown in Table I. Samples 316 and 325 have a much higher total carotene content than any of the other samples listed in Table I. These samples were collected at the same time and differ from No. 321 only in that they were blanched with steam for 7 and 10 minutes, respectively, before drying. It has been pointed out by the authors (12) that steam blanching stabilizes the carotene in fresh alfalfa, so that the usual loss which accompanies drying is avoided. The percentage of  $\beta$ -carotene is somewhat greater in the blanched samples than in the corresponding unblanched sample. Complete extraction of carotene could be effected from coarsely ground unblanched alfalfa, but a finer grind was necessary for extraction of the blanched material. Excellent results were obtained with all types of samples which had passed through a 40-mesh screen.

The new procedure causes some isomerization, but less than methods which require refluxing. A sample of  $\beta$ -carotene (S. M. A. Corporation) was allowed to isomerize slightly and was then carried through the entire analytical procedure. The

additional isomerization for three such determinations averaged 6%. Recovery tests, carried out at the same time, gave excellent results and agree well with those of Wall and Kelley (13). The magnesia columns cause very little loss of carotene.

The samples must be placed in the dark while standing. As pointed out by Pepkowitz (9), carotene, in petroleum ether, is subject to photochemical destruction in the presence of chlorophyll and acetone. No losses seem to occur if the solutions are protected from light.

Possible procedures for the removal of the acetone after filtration have also been compared. Most of this acetone must be removed at this point in the procedure in order to obtain a good separation of pigments on the column. The acetone may be removed by washing with water or by evaporation. Since heating on a steam cone caused only a slight increase in per cent of isomerization as compared with evaporation under reduced pressure or washing with water, this procedure has been followed because of its convenience. Data obtained by these three methods for the removal of acetone are recorded in Table III for comparisons. These data differ from the data recorded in Table I because the analyses were made after prolonged storage.

The acetone present in the final solution need not be removed for spectrophotometric estimation of the  $\beta$ -carotene-neo- $\beta$ -carotene B system. The concentration of acetone in this solution varies from 2 to 4%. This amount of acetone has no effect on the absorption spectra of  $\beta$ -carotene in the range used for carotene estimation. The two curves are identical in the range from 380 to 510 m $\mu$ . The specific absorption coefficients at the critical wave lengths, as obtained by Beadle and Zscheile (1) in hexane, and the authors' measurements in hexane, redistilled Skellysolve B, and a mixture of redistilled Skellysolve B and 5% acetone, are shown in Table IV. Acetone does exhibit considerable absorption at lower wave lengths and would have to be removed if measurements in this range were desired.

While isomers of  $\beta$ -carotene, other than the neo- $\beta$ -carotene of Beadle and Zscheile, have been found in alfalfa, the percentage of  $\beta$ -carotene as calculated by their method has been regarded as a measure of the extent of change in the nature of the carotenoids present. This is considered significant because of the probable reduced nutritional value of the isomeric pigments. Kemmerer and Fraps (4) have recently reported that neo- $\beta$ -carotene B has about one half the vitamin A activity of  $\beta$ -carotene. They have also reported (5) another constituent of the carotenoid fraction of plant materials which is not separated from  $\beta$ -carotene on a magnesia column, but which can be separated on a column of calcium

Table III. Effect of Different Procedures for Removal of Acetone from Carotene Extracts

Sample No.	Steam Cone		Method of Removal of Acetone			
	Total carotene	$\beta$ -Carotene	Vacuum Evaporation		Water Wash	
	Mg./100 g.	%	Total carotene Mg./100 g.	$\beta$ -Carotene %	Total carotene Mg./100 g.	$\beta$ -Carotene %
325	36.7	89	36.4	89	36.4	89
	36.6	87	36.1	90	37.0	89
	36.6	89	36.1	90	35.6	89
331	10.4	81	10.8	82	10.3	80
	10.4	79	10.7	83	10.2	82
	10.7	80	10.8	83	10.3	77
341	19.7	85	19.6	85	19.5	85
	19.5	84	19.3	88	19.4	83
	19.5	85	19.8	87	19.9	81

Table IV. Specific Absorption Coefficients of  $\beta$ -Carotene

Solvent	Wave Length			
	436.0 m $\mu$	450.0 m $\mu$	466.0 m $\mu$	478.0 m $\mu$
Hexane <sup>a</sup>	196	258	206	228
Hexane	198	259	210	231
Redistilled Skellysolve B	193	253	203	224
5% acetone in Skellysolve B	193	253	205	224

<sup>a</sup> Coefficients as given by Beadle and Zscheile (1).



hydroxide. They have identified this pigment as the neo- $\beta$ -carotene U of Polgár and Zechmeister (11). They report that this carotenoid has no vitamin A activity. It may, therefore, become necessary to calculate the carotene composition on the basis of more than two components. This may be possible by the use of spectrophotometric data obtained at other critical points on the absorption curve of the extract. It is hoped that a continuation of spectral studies will furnish data that will be useful in this connection.

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## Determination of Citric Acid in Fermentation Media and Biological Materials

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Several modifications have been made in the colorimetric pentabromoacetone method for the determination of citric acid as proposed by Pucher, Vickery, and associates. The time required per sample

is reduced, while accuracy has not been diminished. This method has been applied to the determination of citric acid in fermented media and tissue extracts with satisfactory results.

CERTAIN strains of the mold, *Aspergillus niger*, convert a large proportion of carbohydrate substrates to citric acid. With most strains of this organism some oxalic acid is also produced. In studying the factors affecting the production of citric acid by this mold (3), it was necessary to have rapid and reasonably accurate methods for the determination of these two acids in the presence of each other.

Since both acids have rather insoluble calcium salts, these salts have been used for their determinations in fermentation media by Doelger and Prescott (2), Wells, Moyer, and May (3), and others. Calcium oxalate is much less soluble than calcium citrate in cold, slightly acid solution, and the two salts may be separated in this manner. Calcium citrate is insoluble in hot neutral solutions. These two acids are usually the main acidic products of this fermentation, and for routine work the difference in acidity between the total acid and that due to oxalic acid may be considered to be due to citric acid. This is a somewhat uncertain procedure, as the mold produces many other acids, and an independent method for the determination of citric acid is desirable.

The volumetric method of Wilkinson, Siphard, Fulmer, and Christensen (9) involves preliminary precipitation of the lead salts of the oxalic and citric acids. The acids are then regenerated and titrated with alkali and ceric sulfate. This method is not very specific for citric acid, as many fermentation acids form rather insoluble lead salts.

A more specific method for the determination of citric acid depends upon its conversion to pentabromoacetone, which may be estimated gravimetrically or colorimetrically. This conversion is based on the fact that when citric acid is oxidized with potassium permanganate in the presence of bromine, under controlled conditions, the acid is converted quantitatively to pentabromoacetone. Deysher and Holm (1) have discussed the diffi-

culties in the gravimetric determination of this derivative of citric acid.

Pucher, Vickery, and associates (4, 5, 6) have estimated the pentabromoacetone colorimetrically by a method based on the yellow color formed by the addition of pentabromoacetone to a sodium sulfide solution. This colorimetric method is somewhat involved and requires quantitative extraction of the pentabromoacetone from the reaction solution. Purinton and Schuck (7) have modified the original method slightly but still require quantitative extraction of the pentabromoacetone and several other rather involved procedures. In the modification described, no attempt has been made to extract the pentabromoacetone quantitatively from the reaction solution. Instead, advantage is taken of the distribution coefficient and a single extraction is made. A single extraction has been found to remove a constant amount of the total pentabromoacetone in any series of samples of uniform volume. This modification has made possible the development of a more convenient procedure.

## EXPERIMENTAL

**REAGENTS.** Sulfuric acid, equal volumes of 95% sulfuric acid and water. 1 *M* potassium bromide, bromine water (saturated), 3% hydrogen peroxide, petroleum ether (acid-washed Skellysolve B).

Dioxane-water mixture, equal volume of dioxane and water. Sodium sulfide solution, 4 grams of sodium sulfide nonahydrate per 100 cc. of solution.

1.5 *N* and 0.1 *N* potassium permanganate.

**PROCEDURE.** If the samples are known to contain reducing material, aliquots preferably containing less than 25 mg. of citric acid are placed in 2.5 × 20 cm. (1 × 8 inch) Pyrex test tubes and 2 cc. of the sulfuric acid solution are added. The total volume is adjusted to about 20 cc. and the samples are boiled for a few minutes. The solutions are then cooled and 3 to 5 cc. of bromine water are added. After 10 minutes, any precipitate



Table I. Recovery of Added Citric Acid from Solutions

Citric Acid Added per Sample Mg.	Solution A <sup>a</sup>		Solution B <sup>b</sup>		Solution C <sup>c</sup>	
	Citric acid found Mg.	Recovery of added acid %	Citric acid found Mg.	Recovery of added acid %	Citric acid found Mg.	Recovery of added acid %
0	0	.....	0.35 0.37	.....	0.24 0.25	.....
			Av. 0.36		Av. 0.24	
0.20	0.19 ...	95.0 100.0	0.57 0.56	105.0 100.0	0.44 0.43	100.0 95.0
0.40	0.39 0.41	97.5 102.5	0.75 0.74	97.5 95.0	0.63 0.65	97.5 102.5
0.80	0.78 0.80	97.5 100.0	1.18 1.16	102.5 100.0	1.04 1.07	100.0 103.8
1.00	1.01 1.02	101.0 102.0	1.36 1.37	100.0 101.0	1.25 1.24	101.0 100.0
1.20	1.23 1.19	102.5 99.2	1.56 1.58	100.0 101.7	1.44 1.47	100.0 102.5
1.60	1.58 1.59	98.8 99.4	1.95 1.99	100.0 102.5	1.85 1.88	100.6 102.5
1.80	1.79 1.77	99.4 98.3	2.10 2.17	96.3 100.6	2.00 2.06	97.8 101.1

<sup>a</sup> Citric acid added to distilled water.<sup>b</sup> Citric acid added to fermented synthetic medium.<sup>c</sup> Citric acid added to fermented molasses medium.

that has formed is removed by centrifugation. The supernatant liquids are decanted off, and adjusted to known volumes. If the samples do not contain appreciable amounts of reducing material they may be adjusted to known volumes without this preliminary bromine treatment.

Aliquots of these solutions, preferably containing 0.2 to 1.8 mg. of citric acid, are placed in test tubes (the 18 × 150 mm. size is convenient) and 0.3 cc. of the sulfuric acid, 0.2 cc. of the potassium bromide, and 1 cc. of the strong potassium permanganate solutions are added. The total volumes are adjusted to about 5 cc. and the tubes are allowed to stand for 5 minutes at room temperature. At the end of this period they are chilled in an ice bath, and the excess permanganate is decolorized with the hydrogen peroxide solution. Care must be taken to keep the reaction mixtures below 5° C. during this step. Any excess peroxide is removed with the weak permanganate. The total volumes are then adjusted to 10 cc. (the test tubes should have a 10-cc. calibration mark for this purpose) and 13 cc. of the petroleum ether are added. The tubes are stoppered, shaken vigorously, and centrifuged (to break any emulsion that might be formed).

Colorimeter test tubes are prepared containing 5 cc. of the water-dioxane mixture and 5 cc. of the sodium sulfide solution, and 10-cc. portions of the petroleum ether extract containing the pentabromoacetone are added. The colorimeter tubes are then stoppered, shaken vigorously, and centrifuged. The color produced should be a light yellow and will be fully developed in 5 minutes. It is stable for several hours. The absorption is determined in a photoelectric colorimeter at 450 mμ. Light absorption by the solution has been found to be reasonably constant from 400 to 450 mμ. A tube containing no citric acid, but which has gone through the same procedure, is used as a 100% transmission standard. At least two known samples of citric acid should be run with each set of analyses. The color follows Beer's law, as is shown in Table I, column 3, and the standards are used to calculate  $k$  in the equation  $\log T = kc$ .

If too large a sample of citric acid has been used, a second smaller aliquot of the petroleum ether extract may be taken, thus avoiding another complete analysis.

As is shown in Table I, the method gives satisfactory results when between 0.2 and 1.8 mg. of citric acid is present in the sample. Larger samples of citric acid may be used. In such cases larger volumes of petroleum ether for extraction of the pentabromoacetone, or smaller aliquots of the petroleum ether extract containing the pentabromoacetone, may be used. However, several known solutions must always be determined in exactly the same manner as the unknown samples.

Recoveries of added citric acid from two types of fermentation media are also shown in Table I. Solution B contained citric acid, oxalic acid, and unfermented sucrose as the major organic constituents. The solution had been prepared for analysis by

removing the mycelium, and adjusting the fermented liquor to a known volume. Various amounts of citric acid were added, as shown in Table I, to an aliquot of this solution corresponding to 0.02 cc. of the original fermented liquor. The same procedure was repeated with solution C, a partially fermented beet molasses medium.

This method has also been applied to the determination of citric acid in tissue extracts. Quantitative recoveries were obtained when citric acid was added to muscle extracts and human seminal fluid. Isocitric acid, *cis*-aconitic acid, *trans*-aconitic acid, and oxalacetic acid do not interfere with this method (Table II) when present in biological samples. Gluconic acid, which is often found in media fermented by *A. niger*, does not interfere. Pucher *et al.* (6) list several other acids often found in fermentation media which are not converted to pentabromoacetone under the conditions of this method and thus do not interfere with the determination of the citric acid.

The following critical points have been noticed:

An excess of hydrogen peroxide in the solution before the petroleum ether extraction leads to low recoveries. The presence of excess potassium permanganate leads to high recoveries.

The solution must be thoroughly chilled before excess permanganate is removed; otherwise, recoveries are erratic.

Some stabilizing agent must be present to stabilize the colored reaction product of the pentabromoacetone and the sodium sulfide. Both 50% dioxane-water and 50% pyridine-water solutions have proved satisfactory.

Interfering materials may be removed from the petroleum ether by acid washing.

The pentabromoacetone should not be allowed to remain in the petroleum ether for more than 15 minutes.

Table II. Specificity of Method

Substance Tested	Maximum Sample Used Mg.	Equivalent of Citric Acid Found Mg.
Isocitric acid	18.8	<0.01
<i>cis</i> -Aconitic acid	1.74	<0.01
<i>trans</i> -Aconitic acid	1.74	<0.01
Oxalacetic acid	6.0	<0.01
Gluconic acid	5.0	<0.01

## DISCUSSION

The advantage of these modifications over the method as originally proposed by Pucher *et al.* (4) are: (1) All manipulations are done in two or at most three test tubes. (2) The extraction procedure which required the use of separatory funnels and the repeated extraction has been simplified. (3) The technique does not have to be rigidly standardized, as several known samples are included with every set of analyses.

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# Analysis of Acetylsulfanilyl Chloride by the Karl Fischer Reagent

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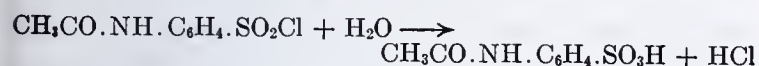
A method is presented for the analysis of acetylsulfanilyl chloride (an intermediate in the production of sulfonamide drugs) for both acetylsulfanilyl chloride content and moisture. The former is obtained by reacting the sample with water in pyridine, measuring the unreacted water by means of the Karl Fischer reagent, and calculating, by difference, the water which has reacted. This is corrected for the water present in the sample, which also reacts. The water in the sample is determined by a titration in methanol with the Fischer reagent in the absence of pyridine. The water determination has an accuracy represented by a standard deviation of  $\pm 0.012\%$  of water. The precision of the acetylsulfanilyl chloride determination is represented by a standard deviation of  $\pm 0.18\%$  of acetylsulfanilyl chloride, with probably no systematic error. The effect upon the values of such impurities as acetone in the methanol reagent, sulfanilic acid in the sample, and other substances is discussed. The method may be applicable to other acid chlorides.

ACETYLSULFANILYL chloride has assumed importance as an intermediate in the production of sulfonamide drugs, where it is used to form acetylated sulfonamides by condensation with various amines:



In this connection, it became desirable to develop a method for determining both the acetylsulfanilyl chloride and the moisture content of the intermediate.

The development of the Karl Fischer reagent as an almost specific reagent for the determination of water (2, 3, 4, 6, 11) has been a noteworthy achievement. This reagent has not merely been used to determine the water content of substances, but has been applied to the determination of many classes of compounds which will react either with water or with appropriate reagents to liberate water. The amount of substance can thus be determined by measuring with the Fischer reagent the loss or gain of water in the reaction mixture. In this way, substances such as alcohols (1), organic acids (7), acid anhydrides (12, 13), and carbonyl compounds (8) have been analyzed. Since sulfonyl chlorides, such as acetylsulfanilyl chloride, can react with water



it seemed reasonable to suppose that the Fischer reagent could be used for the analysis. It was found that the sample could be treated with a solution of water in pyridine, the water content of which had been found by the Fischer reagent, and the consumption of water determined by a titration with the Fischer reagent. Acetylsulfanilyl chloride may, however, contain varying amounts of free water, and so it is necessary to correct for its presence. When the sulfonyl chloride was dissolved in methanol, the water reacted with the chloride only to a slight extent (for which a correction could be applied), thus permitting determination of the water. This agrees with Schroeter's (9) observation that the chloride can be precipitated essentially unchanged from ethanol by addition of water. The authors have confirmed this observation, using methanol instead of ethanol. The obvious explanation of the difference in reactivity of the

sulfonyl chloride with water in the presence or absence of pyridine is that the pyridine acts as a necessary intermediary by removing the hydrogen chloride formed in the reaction.

This work was restricted to analysis of the acetylsulfanilyl chloride, because of specific interest in that compound, but it would seem a reasonable expectation that other acid chlorides, including sulfonyl chlorides, could be analyzed in a similar manner. (After this paper was written, the authors learned that a method had been reported, 10, for determining acetyl chloride by hydrolysis, employing the Fischer reagent.)

## METHOD OF ANALYSIS

REAGENTS. The Karl Fischer reagent is prepared according to the directions of Smith, Bryant, and Mitchell (11) and is dispensed from an automatic buret provided with tubes of Indicating Grade activated alumina to protect it from access of moist air. It is standardized by weighing 2 or 3 drops of water from a Lunge pipet into 10 ml. of anhydrous methanol and titrating with the Fischer reagent. (In this and in the following titrations the end points were observed visually.) From the difference between the volumes of reagent consumed in this titration and by the anhydrous methanol, the weight of water (grams) equivalent to 1 ml. of the reagent,  $T$ , may be calculated.

This laboratory has found this method preferable to standardization through a solution of water in methanol. There is less chance of error because of the possible danger of changes in the water content of a standard water solution. The two methods of standardization are equally precise, being equal to a standard deviation of about  $\pm 0.0035$  mg. of water per ml. of reagent, or about  $\pm 0.1\%$  of the standardization value.

The methanol used must be of the highest quality in respect to freedom from acetone, since the presence of acetone causes errors, discussed below.

A standard solution of water in pyridine is prepared, to contain close to 0.015 gram of water per ml. of solution. This solution is dispensed from an automatic buret protected from access of moisture from the air, and 10-ml. portions with 25 ml. of methanol added are titrated with the Fischer reagent in order to get the exact water content. Let  $E$  = ml. of Fischer reagent for 10 ml. of the pyridine solution plus 25 ml. of methanol. The water content of the reagent will not change if the buret is well protected, except in so far as the water content of the methanol changes and the volume of the pyridine solution alters because of temperature changes. Under normal conditions and except for work of the most extreme accuracy, the change due to this cause may be neglected.  $E$  will change with any deterioration in the Fischer reagent itself, and must, therefore, be checked frequently.

ANALYSIS OF SAMPLE FOR ACETYLSULFANILYL CHLORIDE. From a glass-stoppered weighing bottle, weigh by difference 1 to 2 grams of the sample into a dry 125-ml. Erlenmeyer flask, which is stoppered at once with a two-hole rubber stopper. One hole is fitted with a drying tube, while the other fits over the tip of the buret. Cool the flask containing the sample in an ice bath in order to prevent excessive heating upon addition of the pyridine-water solution. (Such overheating causes condensation of moisture on the upper part of the flask and spoils the analysis.) With the flask in the ice bath, introduce 10 ml. of the pyridine-water solution, stopper the flask immediately, and swirl gently to dissolve the sample completely. Keep the flask in the ice bath only long enough to prevent the temperature from rising. Set it aside after complete solution of the sample for 10 minutes from the time the pyridine-water solution is added. The temperature of the solution at the end of this period should be only slightly below room temperature to avoid condensation of moisture in it when the flask is opened to introduce, from a protected automatic buret, 25 ml. of the methanol which has been used in determining  $E$ . (The methanol is added to dilute the yellow color of the solution in order to avoid difficulty with the end point.) Allow the methanol to wash down the side of the flask and immediately titrate with the Fischer

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reagent to obtain  $F$ , the number of milliliters of Fischer reagent required for the water left unconsumed by the sample. In order to ensure the presence of sufficient water to hydrolyze the sample completely,  $F$  should be greater than the blank for 25 ml. of methanol. Otherwise repeat the determination with a smaller sample.

**ANALYSIS OF SAMPLE FOR WATER.** To a well-dried 125-ml. Erlenmeyer flask rapidly add from a weighing bottle 2 to 2.5 grams of the sample. Minimize exposure of the sample to the air. Keep the flask well stoppered except when adding reagents or titrating, and then substitute for the solid stopper a stopper containing two openings—one provided with a drying tube, the other for introduction of a buret tip. From an automatic buret protected from moisture, add 25 ml. of absolute methanol. Swirl the mixture to get complete solution of the sample and immediately titrate rapidly with the Fischer reagent to the first end point which persists for at least 1 minute. Call this volume  $K$ . Obtain a titration value for the 25 ml. of methanol. Call this volume  $L$ .

**CALCULATIONS.** There is a slight consumption of water by the sample during this procedure which is proportional to the total amount of water present (including that in the methanol) and must be corrected for. The total number of grams of water in the titrated solution equals  $KT$ . The correction to be added to the water content of the sample, expressed as grams of water, equals  $47.3 \times 10^{-3}KT - 0.298 \times 10^{-3}$ . (The derivation of this correction is described below.) If the total water found is greater than 25 mg., the correction factor is  $50.6 \times 10^{-3}KT - 0.337 \times 10^{-3}$ .

Let the % water in the sample equal  $M$ .

$$\text{Then } M = \frac{KT + 47.3 \times 10^{-3}KT - 0.298 \times 10^{-3} - LT}{\text{weight of sample in grams}} \times 100$$

$$= \frac{T(1.0473K - L) - 0.298 \times 10^{-3}}{\text{weight of sample in grams}} \times 100$$

The consumption of water from the pyridine-water reagent by the sulfonyl chloride (equivalent to  $E - F = G$ ) is less than the total amount of water consumed by the amount of water present in the sample itself, which is also consumed in the reaction. Let the percentage of acetylsulfanilyl chloride, uncorrected for the water in the sample, equal  $H$ . Then

$$H = \frac{GT \times 233.67 \times 100}{\text{weight of sample in grams} \times 18.016}$$

where 233.67 = the molecular weight of acetylsulfanilyl chloride and 18.016 = the molecular weight of water. Or

$$H = \frac{12.97GT}{\text{weight of sample (grams)}}$$

$H$  must be corrected for the water in the sample by means of the expression:

$$\% \text{ acetylsulfanilyl chloride} = H + 12.97M$$

#### CORRECTION FOR WATER CONTENT

It was suspected that a slight reaction took place between the acetylsulfanilyl chloride and the water contained in it when the sample was dissolved in alcohol to determine the water content. The extent of this error was determined by adding water and determining how much could be found by analysis.

Acetylsulfanilyl chloride was recrystallized twice from chloroform, washed with petroleum ether, and dried under vacuum over sodium hydroxide and paraffin. The water content (uncorrected) of this sample was 0.019% at the start of the series of experiments and 0.036% at its conclusion. Methanol was dried according to the method of Lund and Bjerrum (5) by refluxing with magnesium and iodine and then distilling until successive cuts gave a constant titration with the Fischer reagent. The methanol which was used had a blank value that varied between 1.1 and 1.4 mg. of water per 25 ml.

In order to conform as closely as possible to the actual conditions of the water determination with respect to the time allowed for reaction of the sample with the water in the solution, the tests were carried out as follows: The weighed sample in a stoppered flask was placed near a weighed Lunge pipet containing water and also near the automatic buret containing the methanol. Twenty-five milliliters of methanol were added to the sample, the mixture was swirled for a few seconds to effect solution, and some water was immediately added from the Lunge pipet (which was equipped with a rubber stopper after

weighing in order to minimize absorption of water from the air by the methanol during the addition of water from the pipet). The contents of the flask were immediately titrated with the Fischer reagent and finally the Lunge pipet was reweighed to determine the exact weight of water added.

Table I gives the data from these determinations. Column 2 is the sum of the water present in the methanol and in the sample (as determined by the Fischer titration) and of the added water.

The values in columns 2, 3, and 4 would seem to indicate some correlation between the water lost and the total water found or present. On the assumption that this correlation could be expressed as a linear function and that column 3 contains the independent variable, the best linear equations were calculated (by the method of least squares) using all 21 values in one case, and only the first 17 values in the other case. The latter equation was calculated because usually the determinations have involved a total water content of less than 25 mg.

Correction in grams of water =

$$50.6 \times 10^{-3} \times \text{grams of water found} - 0.337 \times 10^{-3} \quad (1)$$

Correction in grams of water =

$$47.3 \times 10^{-3} \times \text{grams of water found} - 0.298 \times 10^{-3} \quad (2)$$

Table I. Determination of Error in Analysis for Water

Acetylsulfanilyl Chloride Added	Total Water Present	Total Water Found	Water Lost (Present Minus Found)
Grams	Mg.	Mg.	Mg.
2.594	3.0	3.0	0.0
2.589	3.3	3.0	0.3
2.096	3.3	3.8	-0.5
2.496	6.1	6.3	-0.2
2.717	7.5	7.7	-0.2
2.277	8.3	8.2	0.1
2.721	12.5	12.2	0.3
2.021	12.7	12.4	0.3
2.432	13.3	13.1	0.2
1.961	15.2	14.6	0.6
1.770	15.6	15.2	0.4
2.163	15.9	15.3	0.6
1.927	16.0	15.4	0.6
2.075	18.2	18.0	0.2
2.358	20.8	19.9	0.9
2.464	22.8	22.1	0.7
1.974	25.5	24.7	0.8
1.952	33.3	32.4	0.9
2.263	44.4	42.3	2.1
2.173	60.1	57.0	3.1
2.098	71.1	68.4	2.7

#### PRECISION AND ACCURACY

The deviations of the values in column 4 of Table I from the corresponding values calculated from Equations 1 and 2, obtained by substituting in the equations the appropriate values in column 3, measure the precision of the water determination. This also measures the accuracy, provided one neglects the slight error due to the fact that a part of each value in column 2 is obtained by an uncorrected titration of the water present in the sample; but since the latter is low, this error is negligible.

Using Equation 1, the average deviation (for all the 21 values in Table I) is  $\pm 0.20$  mg. of water (the maximum deviations being  $+0.57$  and  $-0.40$  mg.) and the standard deviation (root mean square) is  $\pm 0.27$  mg. of water. This would be equivalent to  $\pm 3.5$  mg. of acetylsulfanilyl chloride and, for an average sample weight for the water determination of 2.1 grams, it would amount to  $\pm 0.17\%$  of acetylsulfanilyl chloride.

Using Equation 2, for 17 values (with maximum deviations of  $+0.46$  and  $-0.38$  mg.), the average deviation is  $\pm 0.17$  mg. of water and the standard deviation is  $\pm 0.22$  mg. of water. The equivalent values for acetylsulfanilyl chloride would be  $\pm 2.9$  mg. or  $\pm 0.14\%$  of acetylsulfanilyl chloride.

From duplicate analyses of 17 actual samples, a standard deviation from the respective means of  $\pm 0.23$  mg. of water was calculated; or, for a 2-gram sample,  $\pm 0.012\%$  of water. From duplicate analyses for acetylsulfanilyl chloride on the same 17 samples, a standard deviation of  $\pm 2.3$  mg. of acetylsulfanilyl



chloride was calculated; or, for an average sample weight of 1.3 grams,  $\pm 0.18\%$  of acetylsulfanilyl chloride. There is no reason to believe that the analysis has any systematic error, but because of the difficulty of preparing a sample of known purity this cannot be stated with certainty. Some samples have, however, been analyzed which gave values not far from 100%.

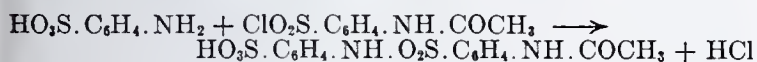
#### EFFECT OF VARIATIONS IN TIME OF HYDROLYSIS

The method calls for a period of 10 minutes after the addition of the pyridine-water solution for the completion of the hydrolysis. Actually, the reaction is much more rapid. A series of determinations was made on a single sample in order to test this point. In order to separate the time variable from that which would be introduced by the presence toward the end of the reaction of variable concentrations of Fischer reagent, the volume of pyridine-water solution was varied according to the size of the sample (since it was not convenient with a substance like acetylsulfanilyl chloride to keep the sample weight constant), so that the back-titration with the Fischer reagent would be fairly constant. The values obtained, all of which agreed within the experimental error of the method, showed that it is immaterial whether the hydrolysis is allowed to proceed for 3 or 25 minutes.

#### EFFECTS OF IMPURITIES OTHER THAN WATER

The Fischer reagent is specific for water, with the exception that certain inorganic oxides will also be titrated (6) and occasionally a substance may be met with, such as hydroxylamine (8), which will react and so will require special treatment to avoid interference. In acetylsulfanilyl chloride, some possible impurities may be acetylsulfanilic acid, hydrochloric acid, acetic acid, sulfanilic acid, and water. Obviously, neither acetylsulfanilic acid nor hydrochloric acid interferes in the determination of acetylsulfanilyl chloride because they are products of the reaction, and the reaction has been shown to proceed as expected with recrystallized material. No additions of these two substances were made to test their effect upon the determination of water, but no difficulty was obtained in analyzing for water in rather impure samples which would contain these substances if they were normal impurities. A method has been reported for determining acetic acid by its esterification with methanol (7) to liberate water, but since this requires a boron trifluoride catalyst, it does not interfere here.

Sulfanilic acid does not introduce any error into the water determination. It does introduce an error into the acetylsulfanilyl chloride determination. Presumably, it reacts with the compound as follows:



thus preventing an equivalent quantity of the chloride from reacting with the water.

Table II gives data showing the quantities of the sulfonyl chloride which have been consumed by varying amounts of added sulfanilic acid. The ratio of chloride consumed to sulfanilic acid present averages 0.99, with considerable variation of the individual ratios from this average. The variations from the 1 to 1 ratio are probably not indicative of a real departure from the stoichiometric proportions, but point to certain experimental difficulties. For example, the mixtures with small amounts of sulfanilic acid might show variations because of the low equivalent net volume of Fischer reagent for the sulfanilic acid. In other cases, the difficulty may be that the sulfanilic acid, which is added as a solid, dissolves more slowly than the sulfonyl chloride in the water-pyridine solution, so that the chloride may react preferably with the water. There is also additional exposure to atmospheric moisture.

The sulfanilic acid present may be determined by acidification and titration with sodium nitrite solution at room temperature.

Table II. Effect of Sulfanilic Acid

(1) Sulfanilic Acid Added Millimole	(2) Acetyl- sulfanilyl Chloride Taken Millimoles	(3) Acetyl- sulfanilyl Chloride Found Millimoles	(4) Acetyl- sulfanilyl Chloride Consumed (2) - (3) Millimole	(5) Ratio (4) to (1)
0.0583	7.006	6.942	0.064	1.10
0.1243	5.853	5.742	0.111	0.89
0.1942	4.243	4.099	0.144	0.74
0.4131	5.079	4.651	0.428	1.04
0.6390	5.923	5.189	0.734	1.15
0.6674	4.380	3.690	0.690	1.03
				Av. 0.99

A correction for the sulfonyl chloride consumed may then be applied. All the samples examined by the authors had so little sulfanilic acid that the possible variations in the stoichiometric ratio just mentioned were of no practical significance.

Another impurity which may cause difficulty is acetone in the methanol reagent.

A sample of chloride was analyzed for water, using in one set of determinations methanol containing 0.003% of acetone, and in the other set methanol containing 0.34%. The first set gave a value of 0.06% and the second set a value of 0.50% of water in the chloride. Another sample was analyzed in the same manner, in one case using methanol with only a trace of acetone in it and in the other the same methanol to which 0.35% of acetone had been added. The latter methanol solution gave a value of 0.79% water in the chloride in comparison with a value of 0.13% water obtained using the pure methanol.

Acetone may interfere with the Fischer titration for water in alcohol because of ketal formation, which causes the liberation of water. However, addition of acetone to methanol in the concentrations mentioned did not cause an increase in the apparent water value of the methanol. There would seem to be some specific effect of the chloride involved, possibly in catalyzing ketal formation. Since a blank value would thus not correct for this effect, the presence of any but the merest trace of acetone in the methanol will lead to high water values for the chloride, with a consequent high value for chloride.

#### ACKNOWLEDGMENTS

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# Osmometry of High-Polymer Solutions

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A glass osmometer of simple design is described which is especially useful for osmotic pressure measurements of high-polymer solutions that tend to form a stable foam. The assembly and operation of the osmometer are described and data are presented to illustrate the order of reproducibility obtained.

THE determination of the osmotic pressure of high-polymer solutions is, at present, the most feasible method of measuring the colligative properties of these systems, since ultracentrifuge equipment is very expensive and its use is accordingly restricted.

The osmotic pressure of a solution may be determined by either the static-elevation method, which involves the measurement of the liquid head developed by the influx of solvent into the solution through a suitable semipermeable membrane by the operation of osmotic forces arising from the difference in the activities of the solvent molecules in the two phases, or the dynamic-equilibrium method, in which the magnitude of an externally applied pressure necessary to counterbalance the osmotic pressure is determined.

While it is possible to use either method or a combination of them with any osmometer, the designs of the various instruments described in the literature are sufficiently different, depending upon which method is to be used, to permit classifying them as static-elevation osmometers (1, 4, 7, 8, 12, 14, 22, 23, 24) and dynamic-equilibrium osmometers (2, 3, 5, 6, 11, 13, 15, 21, 25). The principal advantage of the dynamic method is the rapidity with which a measurement can be made. In order to obtain this advantage effectively, it is necessary to employ a large membrane, with adequate support to minimize "ballooning" effects. This requires a relatively complex instrument, involving specially milled channels, metal-to-glass seals, needle valves, stopcocks, etc. These latter factors increase the possibility of leaks and the entrapment of air, especially if the solution being measured has a tendency to form a stable foam.

The static-elevation instrument avoids most of these difficulties because of its inherent simplicity of design and of operation. The disadvantage of the longer period of time required to complete a single determination can be mitigated by proper selection of instrument dimensions and, since this type of instrument is compact and inexpensive, by operating a number of them simultaneously, using solutions of different concentration or of different polymers.

In Figure 1 is shown a static-elevation osmometer which has been used in this laboratory for the osmometry of a number of high polymers in a variety of solvents. This instrument is a modification of the osmometer described by Schulz (24). The transparency obtained by the use of a heavy-walled glass cell and the use of a glass-stoppered cylinder of sufficient height to enclose the inner assembly completely and assure a vertical position are the principal improvements. An etched scale on the capillary is optional and is suggested as a possible means of avoiding the use of a cathetometer.

### OSMOMETER AND MODE OF ASSEMBLY

The inner assembly (Figure 1) consists of a 4-mm. wall glass cell, *A*, to which the capillary, *B*, of about 0.7-mm. bore is fastened through a 7/25 ground-glass joint. The metal portions of the inner assembly consist of a clamp base, *C*, a clamp yoke, *D*, and their fasteners. Although brass is satisfactory for many systems, it is advisable to use either 18-8 stainless steel or nickel, the latter being preferred. (Specifically, nickel-plated brass and

18-8 stainless steel have been found unsatisfactory when used with aqueous phosphate or phthalate buffers.) The base should be perforated as shown, using a No. 1 drill. The flat membrane of the assembled cell is shown at *E*, resting on a support of ash-free filter paper, *F*.

Before a new cell is put into service, the footing of cell *A* should be inspected for flatness against a piece of plate glass, and, if necessary, ground flat using 1600-mesh emery powder. The glass joint should also be tested for possible leaks. This test is conducted as follows: The joint is wet with acetone and seated immediately. The end of a low-pressure 0.3 kg. per sq. cm. (5 pounds per sq. inch) air hose is held at the place inside *A* where the inner joint of the capillary tube comes through, forcing the hose against the shoulder of the cell. This will tend to displace the liquid seal and will quickly do so if the joint is even slightly leaky. If the joint is satisfactory, the seal will withstand this pressure for 30 to 60 seconds without drying out. If the joint fails to pass this test, it should be ground with 1600-mesh emery powder until it does. It is important that the inner portion of the joint extend beyond the grinding of the outer portion of the joint, if grinding is to produce any improvement. The cell and its capillary should then be marked, so that this particular pair will always be used together.

The process of assembling the osmometer is very simple. A disk of filter paper is cut out or punched out with a hardened steel punch of sufficient size to cover the floor of the clamp base, *C*. The base and the paper are wetted with solvent and a similar disk of previously prepared membrane material is laid on the paper, care being taken not to allow the membrane to become dry. A clean cell is placed on the membrane and the yoke, *D*, is put into place and tightened. It will not be necessary to tighten the yoke screws excessively—a little more than finger-tight will suffice.

The interior parts of the assembly are rinsed several times with the solution and then completely filled with it. Solution is then sucked into the capillary tube until it is about half full and the tube inserted into the glass joint of the cell, the excess solution being allowed to flow around the unseated joint and not through the upper end of the capillary tube. The final position of the capillary meniscus can be adjusted to any desired level by allowing the solution to flow out around the joint in the manner just

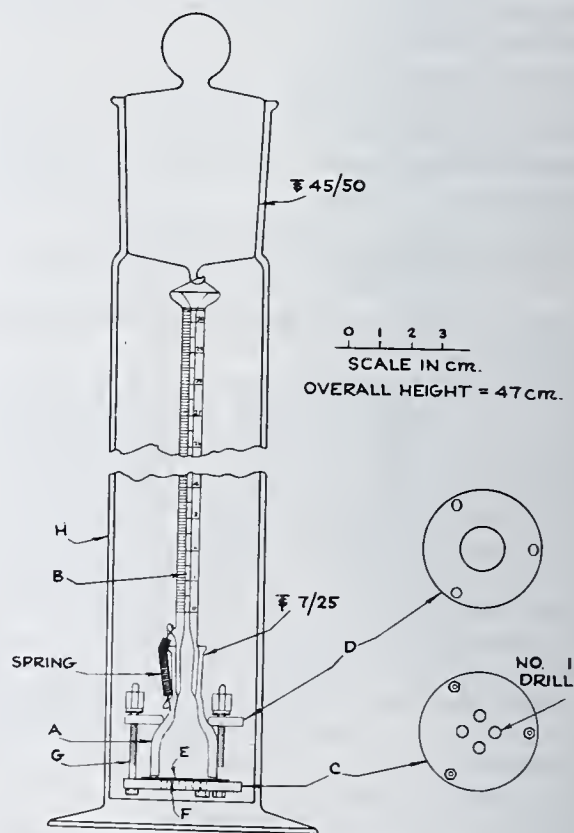


Figure 1. Osmometer



described. (If necessary, the liquid can be forced out by means of a little pressure.) After the desired level is obtained, the joint is firmly seated and secured with a suitable fastener, such as a light spring or rubber band, depending on the solvent. It is important that no air be trapped inside the instrument, and this can easily be avoided after a little experience. No lubricant is used in the joint, for obvious reasons. Pliers whose jaws are covered with sections of medium-walled rubber tubing will be found helpful in seating and unseating the joint (the upper hook, Figure 1, must be sufficiently robust).

The outer surfaces of the assembly are thoroughly washed with solvent to remove all traces of solute and then lowered into the cylinder, *H*, containing about 100 cc. of solvent. This operation will trap some air in the holes of the base, which can easily be removed by raising and lowering the (inner) assembly more or less rapidly while holding the cylinder at an angle of 45 degrees. The level of the external liquid is adjusted to a suitable height and the cylinder closed. In order to obtain a vertical position of the capillary, it is recommended that the inner assembly be suspended from the top of the cylinder. The osmometer should then be thermostated to within  $\pm 0.1^\circ \text{C.}$  of the desired temperature, until no further change in the head of liquid is observed.

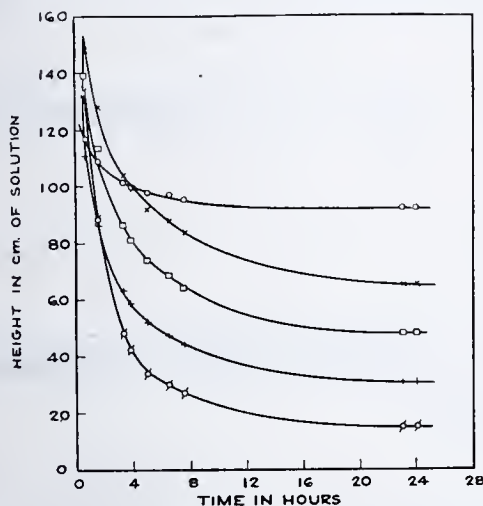


Figure 2. Equilibration Curves at Various Polymer Concentrations

System, polyvinyl alcohol in dilute salt solutions  
Grams/100 ml.

○—○—○. 2.00  
×—×—×. 1.60  
□—□—□. 1.20  
+—+—+. 0.80  
◇—◇—◇. 0.40

It will be found advantageous to adjust the initial positions of the inner and outer levels to as near the anticipated final head of liquid as prior information regarding the systems permits. This procedure reduces the time required for equilibration which, in extreme cases, may amount to from 8 to 24 hours, depending on the temperature, the solvent, the initial head, and the permeability of the membrane. Equilibrium may be approached from either above or below if the solution readily wets glass; solutions composed of nonaqueous liquids, such as acetone, benzene, alcohols, chloroform, etc., are examples. With aqueous solutions, however, it is essential to approach the equilibrium position from above—i.e., the determination should be begun with the inner meniscus near the top of the capillary. This will assure a receding angle of contact at the solution-glass-air interface throughout the determination, which is essential in order to secure the proper correction for capillarity (see below).

It is very important that the glass parts of the osmometer be scrupulously clean in making determinations involving aqueous solutions. The following treatment has been found adequate: All glass parts are immersed in a hot concentrated nitric-sulfuric acid bath (1 to 1 by volume) for several hours or overnight, rinsed with distilled water, and immersed in a warm 20% caustic solution for about 5 minutes. They are then rinsed thoroughly with distilled water after a dilute acid rinse; all contact with organic solvents is avoided for drying. The capillary may be dried by sucking clean, dry air through it.

#### CAPILLARY CORRECTION CONSTANT

The observed height at equilibrium must be corrected for the capillary rise of the solution. This capillary correction constant

is easily determined by a direct measurement, using the capillary (without the cell) and the solution used in the osmotic-pressure determination. The capillary correction constant obtained by use of the pure solvent may be employed if the surface tension of the solution does not differ from it by more than 5% (calculated for a capillary of 0.7-mm. bore). Since this is usually, if not always, the case, it is not necessary to measure this correction for each individual solution. The precautions regarding cleanliness of glass and the direction of approach to equilibrium discussed above apply to the measurement of the capillary correction constant. A receding water-air-glass angle of contact is, in general, more stable and reproducible than the advancing angle, and it is essential, therefore, to base the capillary correction on it.

The relatively unstable water-air-glass interface can be eliminated altogether by a somewhat more complicated procedure using, in the capillary, an immiscible liquid, such as toluene or xylene. A liquid-glass interface is formed in the inner part of the glass joint by first sucking a small quantity of organic liquid into the joint, followed by some of the solution being measured. A little experience will be necessary to get the correct quantity of organic liquid, so that the liquid-liquid interface is in the wide part of the joint and the liquid-air interface is near the top of the capillary. The capillary tube and the cell are then assembled as previously described. The final head of liquid, in pressure units of centimeters of liquid of unit density, will be the algebraic sum of the height-density products of the two liquid columns.

#### MEMBRANES

Regenerated cellulose, which has not been dried out, is probably the most satisfactory material.

Commercial cellophane in this form can be obtained from several manufacturers (Sylvania Industrial Corp., Fredericksburg, Va., and E. I. du Pont de Nemours & Co., Inc., Wilmington, Del.). The material should not be waterproofed and should have a wet thickness (in water) of about 0.1 mm. (0.004 inch). It is important to specify that the material be free of wrinkles. Undried commercial cellophane has been used in the osmometry of such diverse systems as polyisobutylene in cyclohexane (11), polyvinyl acetate in acetone and in benzene, polyvinyl alcohol in water and in dilute salt solutions, and various cellulose esters in acetone (26).

Denitrated collodion may be used, although it is, generally speaking, not so uniform as cellophane. Flory (11) states that denitrated collodion membranes are more permeable than swollen cellophane, although this depends largely on the manner of preparation (9, 10).

Membranes are conditioned for use by washing thoroughly with distilled water to remove all formaldehyde (the stock should be stored in a dilute formaldehyde solution to prevent bacterial action), and the water is then displaced with acetone if the membrane is to be used with organic liquids. The water should be displaced gradually by washing the material in successive aqueous acetone solutions of increasing acetone concentration—e.g., in four solutions of 25, 50, 75, and 100% acetone. This method does not materially alter the permeability of the membrane (20). Pure acetone can be displaced directly with any desired liquid.

Occasionally, a membrane behaves erratically, which is manifested by inconsistently low final pressure results or by an irregular equilibration curve. This is presumably due to some structural anomaly in the material itself. The possibility of solute permeation should not be overlooked, especially if the average molecular weight of the polymer is very low. (Flory, 11, measured polymers with molecular weights as low as 6000, using swollen cellophane membranes. Measurements of polymers of 10,000 to 15,000 have been made in this laboratory.) This can be determined by a suitable test of the external liquid (dialyate) at the conclusion of an osmotic-pressure determination.

#### TREATMENT OF DATA

After the capillary correction constant,  $h_c$ , has been subtracted from the observed equilibrium height,  $h_{obs.}$ , the resultant height is the osmotic pressure of the polymer in centimeters of solution. It is desirable to express this pressure in units which are independent of the density, so that values obtained for various



Table I. Reproducibility of Osmotic-Pressure Determinations

Solute	Solvent	Concentration G./100 cc.	Temperature ° C.	Osmotic Pressure $\pi \times 10^3 \text{ atm.}$
Cellulose acetate (40.42% acetyl)	Acetone	1.00	25	4.50 4.70
Cellulose acetate-butyrate (13.0% acetyl, 37.0% butyryl)	Acetone	1.35	25	6.95 7.05
Cellulose nitrate (11.95% N)	Acetone	1.19	25	10.14 10.32
Cellulose butyrate (55.6% butyryl)	Acetone	1.00	25	5.06 5.02 4.90
Cellulose acetate-phthalate (19.6% acetyl, 30.3% phthalyl)	Acetone-methyl cellosolve (80-20 by volume)	1.00	25	5.08 5.29 5.22
Polyvinyl acetate, RH 361	Acetone	1.00	25	1.92 1.86 1.91
Polyvinyl acetate, Gelva V-60	Acetone	2.00	25	1.92 9.04 9.32
	Acetone	2.50	25	14.70 14.82
Polyvinyl alcohol, RH 393	Water	1.20	25	7.82 8.23 7.76 8.20
Gelatin, lime-processed deashed calf, (B461-48D)	Water	2.00	40	6.57 5.96 6.30
	Water	2.50	40	8.98 8.70 8.70

systems can be compared directly. The pressure in atmospheres,  $\pi$ , can be calculated by using the following equation:

$$\pi = \frac{(h_{\text{obs.}} - h_{\sigma})d_s}{1033} \quad (1)$$

where  $d_s$  is the density of the solution in grams per cubic centimeter, which should be known to about  $\pm 0.005$ . It will be sufficiently accurate to use density values obtained by interpolation from a straight line drawn between the known or measured density of the solvent and the measured density of a solution of approximately the maximum concentration to be studied.

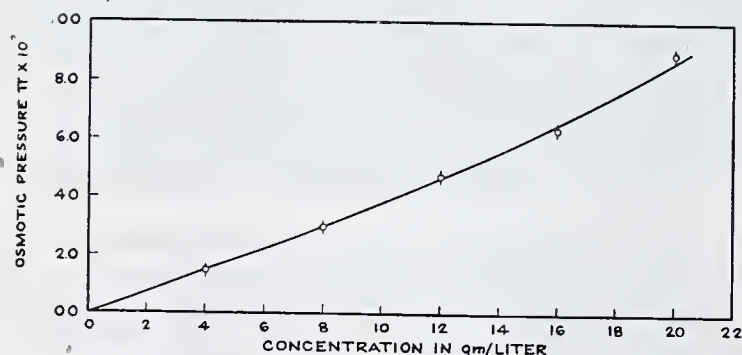


Figure 3. Dependence of Osmotic Pressure on Polymer Concentration  
System, polyvinyl alcohol in dilute salt solution

It is recommended that the osmotic pressure be plotted as a function of the polymer concentration, and the best average curve through these points and the origin then drawn. The pressure values at any desired concentration may then be read off for the calculation of any specific function of pressure and concentration. This process yields a "graphical average", and is more satisfactory in many cases than calculating the function directly from the data.

Probably the most accurate manner of plotting osmotic data

for general high-polymer studies is according to the following equation, suggested by Huggins (17, 19):

$$\frac{\pi}{C_2} - \frac{RTd_1}{3M_1d_2^3} \times C_2^2 = \frac{RT}{M_2} + \frac{RTd_1}{M_1d_2^3} \left( \frac{1}{2} - \mu_1 \right) C_2 \quad (2)$$

where  $\pi$  is the pressure in atmospheres,  $C_2$  is the polymer concentration in grams per cubic centimeter,  $R$  is the gas constant in cubic centimeters atmospheres per degree per mole,  $T$  is the absolute temperature,  $d_1$  and  $d_2$  are the densities of solvent and solute, respectively,  $M_1$  and  $M_2$  are the molecular weights of solvent and solute, respectively, and  $\mu_1$  is a constant depending on the nature of the solvent and solute. The second term on the left in Equation 2 is negligible for many systems, but may be significant in others (17, 18, cf. Figure 4). By plotting the term, or terms, on the left against  $C_2$ , a straight line should be obtained (at least at the lower concentrations), whose intercept is inversely proportional to the number-average molecular weight of the polymer (cf. Equation 3):

$$\lim_{c \rightarrow 0} \left( \frac{\pi}{C_2} \right) \cong \lim_{c \rightarrow 0} \left( \frac{\pi}{C_2} - \frac{RTd_1}{3M_1d_2^3} \times C_2^2 \right) = \frac{RT}{M_2} \quad (3)$$

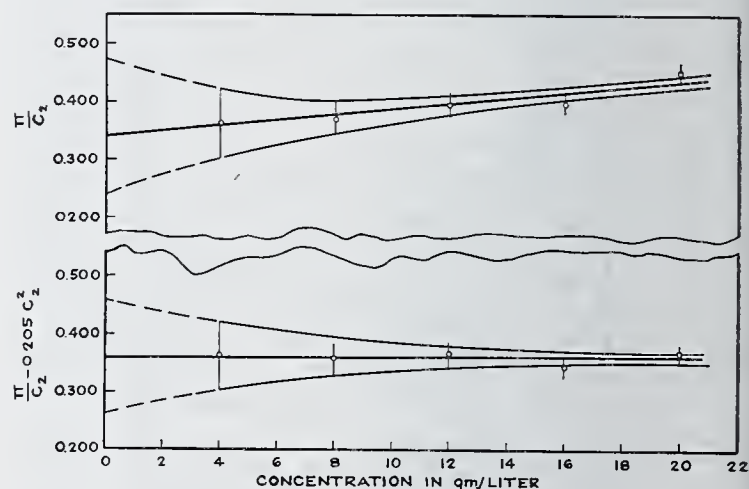


Figure 4. Dependence of Osmotic Pressure-Concentration Ratio (with and without Correction Term) on Polymer Concentration  
System, polyvinyl alcohol in dilute salt solution

For a discussion of the significance of the  $\mu_1$  value, which is related to the slope, reference is made to the original papers of Huggins (16, 18, 19).

#### REPRODUCIBILITY OF EXPERIMENTAL RESULTS

The precision obtained with aqueous solutions is lower than that obtained with organic liquid systems. This is shown in Table I. Aqueous systems usually show an uncertainty of about 2 or 3  $\times 10^{-4}$  atmosphere (2 or 3 mm. of water), which amounts to 10 or 20% of the total pressure in the very low pressure region. The effect of this uncertainty is shown graphically by the vertical lines in Figures 3 and 4. The results obtained with a commercial "high-viscosity" polyvinyl alcohol in dilute salt solution (Du Pont's No. RH 630 in a phosphate buffer solution containing  $1.4 \times 10^{-3}$  mole of sodium monohydrogen phosphate,  $7.8 \times 10^{-3}$  mole of sodium dihydrogen phosphate, and  $1.3 \times 10^{-2}$  mole of sodium chloride per liter. The ionic strength of the "solvent" = 0.025 mole per liter) are collected in Table II and shown in Figure 2 (equilibration curves), Figure 3 (pressure-concentration dependence), and Figure 4 (reduced pressure-concentration dependence) (cf. Equation 2).

Extrapolation of either plot—i.e., with and without the  $0.205 C_2^2$  term—yields an intercept from which may be calculated a number-average molecular weight of 70,000, with an uncertainty of approximately 10%. For similar systems of higher molecular weight, this uncertainty increases rapidly and soon becomes prohibitively large—for example, at  $M_2 = 225,000$ , the uncertainty will amount to more than 50%.



Table II. Osmotic Pressure of Polyvinyl Alcohol, RH 630, in Dilute Salt Solution

$C_2$ Gm./Cc.	$h_{cm.}$ Water	$\pi \times 10^3$ Atm.	$\pi/C_2$	$0.205 \times C_2^2$	$\frac{\pi}{C_2} -$ $0.205 \times C_2^2$
0.0040	1.50	1.45	0.363	0.003	0.360
0.0080	3.06	2.96	0.370	0.013	0.357
0.0120	4.88	4.72	0.394	0.029	0.365
0.0160	6.54	6.32	0.395	0.052	0.343
0.0200	9.27	8.97	0.449	0.082	0.367

$R = 82.1$  cc. atm. per degree per mole.  $T = 298^\circ \text{A}$ .  $d_1 = 1.00$  gram per cc.  $M_1 = 18$ .  $d_2 = 1.62$  grams per cc.

The reproducibility of measurements of nonaqueous polymer solutions is about two to three times better, and therefore a considerably greater range of molecular weight determinations can be obtained with an accuracy of 10% or less.

## ACKNOWLEDGMENT

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## Errors in the Zeisel Methoxyl Values for Pectin Due to Retained Alcohol

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Retained ethanol causes the methoxyl content of pectins, as measured by the Zeisel method, to be as much as 20% higher than saponification values. This ethanol cannot be removed by the usual drying techniques but can be removed by humidification followed by drying. Acetone rather than ethanol precipitation results in good agreement between the two methods of analysis. Retained isopropyl alcohol can be removed by drying at  $100^\circ \text{C}$ .

**DISCREPANCIES** between results obtained by the Zeisel and saponification methods of determining methoxyl in pectin have been observed in this laboratory. The former method has yielded results as much as 20% higher—e.g., a sample analyzed by the two methods showed methoxyl contents of 12.5 and 10.5% by the Zeisel and saponification methods, respectively. An investigation of these differences showed that the disagreement was due to alcohol that could not be removed by ordinary drying procedures but could be removed by humidification prior to drying. When the retained alcohol was removed, the saponification procedure gave results equal to or higher than those by the Zeisel method, the extent of alkali hydrolysis being a function of alkali concentration, time, and temperature.

## METHODS

**SAPONIFICATION PROCEDURE.** The method was a modification of that of Olsen *et al.* (7); stoppered flasks instead of beakers were used in order to decrease the error caused by absorption of carbon dioxide, and the Hinton (4) indicator, which is a mixture

of 3 parts of phenol red to 1 part each of bromothymol blue and cresol red, was used instead of phenolphthalein. A 1-gram sample of pectin was placed in a 500-ml. Erlenmeyer flask, a few milliliters of alcohol were added to wet it, followed by 300 ml. of water, and the mixture was allowed to stand until the pectin dissolved. After the solution had been titrated to the indicator end point with 0.1 *N* sodium hydroxide, 20 ml. of 0.5 *N* sodium hydroxide were added, the flask was stoppered, and the reaction mixture allowed to stand for 2 to 3 hours at room temperature, whereupon 20 ml. of 0.5 *N* hydrochloric acid were added and the solution was back-titrated with 0.1 *N* sodium hydroxide. This last titer corresponds to the saponification value.

**ZEISEL METHOD.** Clark's (1) modification of the Viebock and Schwappach method for the determination of methoxyl and ethoxyl groups was used. This consists in a volumetric determination of the alkyl iodide formed by the action of hydriodic acid on methoxyl and ethoxyl groups.

**HUMIDIFICATION PROCEDURES.** The pectin samples in shallow dishes were placed in a humidifier comprising a desiccator in which water replaced the drying agent. Toluene was kept on the water to prevent mold growth. Humidification of the pectin could be accomplished by allowing the samples to stand in the humidifier or by bubbling a slow stream of air through the water.

## RESULTS

Since it seemed likely that the high Zeisel results were due to ethanol as an impurity in the pectin, an attempt was made to remove the ethanol by drying in an Abderhalden dryer. As can be seen from Table I, this decreased the Zeisel methoxyl content determination to only a small extent.

Mease (5) showed that cotton, wool, silk, and rayon absorb alcohol and hold an appreciable amount of it, even when dried to constant weight at temperatures considerably above the boil-



ing point of alcohol. The alcohol could be removed from the fibers by rinsing in water and again drying. Hilbert and Jansen (2) later found that glucosidocytosine attaches alcohol with such great tenacity that the alcohol could not be quantitatively removed by drying in an Abderhalden dryer at 135° C. at a pressure of 1 mm. or even 10<sup>-5</sup> mm. of mercury. However, when the material was placed in an atmosphere of high humidity, the alcohol was gradually displaced by water, which was then easily removed by the Abderhalden procedure.

This humidification technique was adopted for several pectin samples selected at random. As can be seen from Table II, the technique brought the results by the Zeisel method into agreement with those by the modified Olsen saponification method. The observation that the disagreement in methoxyl values was less than those reported in Tables I and III was to be expected, since some atmospheric humidification had undoubtedly taken place during storage.

Table I. Effect of Abderhalden Drying on Methoxyl Content of Pectins Having Higher Zeisel than Saponification Methoxyl Values

Sample <sup>a</sup>	OCH <sub>3</sub> Before Drying		Loss in Weight on Drying %	OCH <sub>3</sub> After Drying <sup>b</sup>	
	Saponification %	Zeisel %		Saponification %	Zeisel %
1. Citrus, 200 grade, purified	10.5	12.5	5.95	10.5	12.1
	10.6	12.4	....	10.4	12.1
2. Citrus, 185 grade	9.1	9.8	7.27	9.1	9.9
	9.1	9.8	....	9.0	9.6
3. Citrus, 178 grade	10.1	11.6	2.35	10.2	11.2
	10.2	11.7	....	10.3	11.2

<sup>a</sup> Sample 1 purified according to method of Olsen *et al.* (7) including drying in vacuum oven. Sample 3 dissolved, reprecipitated with ethanol, and dried in vacuum oven at 70° C. overnight previous to analysis and Abderhalden drying. Samples 1 and 2 dried in P<sub>2</sub>O<sub>5</sub>-containing Abderhalden dryer at 78° C. at pressure less than 1 mm. of Hg for 8 hours. Sample 3 was dried in dryer at 100° C. to constant weight; total heating time, 8 hours.

<sup>b</sup> Calculated on original moisture basis.

The error due to retained solvent can be avoided not only by use of the humidification treatment but also by the use of solvents for precipitation that are practically inert in the Zeisel methoxyl determination—e.g., acetone—or solvents, such as isopropanol, that are less strongly retained than is ethanol and therefore can be removed in an Abderhalden dryer (Table III). It is evident also from Table III that 2 days' humidification is sufficient to remove retained ethanol. Determinations made after 4 and 6 days' humidification agreed with those made after 2 days. It is to be expected that pectin samples which have been allowed to stand open in a room of high humidity for some time will no longer contain combined alcohol.

Table II. Effect of Humidification on Methoxyl Content of Pectins

Sample	Without Humidification Treatment		With Humidification Treatment <sup>a</sup>	
	Zeisel %	Saponification %	Zeisel %	Saponification %
Apple, 300 grade	6.4	6.0	6.0	6.2
	6.5	6.0	6.1	6.1
Apple, 285 grade	7.8	7.6	7.5	7.4
	7.9	7.5	7.6	7.4
Citrus, 178 grade	11.6	10.1	10.0	10.3
	11.7	10.2	10.0	10.4
Citrus Pectinum NF VII	9.8	9.7	9.3	9.5
	9.8	9.7	9.3	9.5
Citrus, 200 grade, purified	10.3	10.1	9.5	9.5
	10.4	10.1	9.5	9.6

<sup>a</sup> Humidification treatment: Pectin samples in shallow dishes placed in desiccator over water and slow stream of air bubbled through water for one month. Toluene kept on water to prevent mold growth. Samples then dried in a vacuum oven at 70° C. for 24 hours.

Table III. Methoxyl Values for Pectin<sup>a</sup> Precipitated by Acetone, Ethanol, and Isopropanol as Related to Similar Values after Humidification

Solvent Used to Precipitate Pectin from Solution <sup>b</sup>	Humidification Treatment	Methoxyl Content	
		Zeisel %	Saponification %
Acetone	None	10.1	10.2
	None	10.2	10.2
Isopropanol	None	11.1	10.2
	None	11.0	10.3
Isopropanol	2 days	10.0	10.5
	2 days	10.0	10.5
Isopropanol	None	10.1 <sup>c</sup>	....
	Dried at 100° C. in Abderhalden	None	10.1 <sup>c</sup>
Ethanol	None	11.6	10.1
	None	11.7	10.2
Ethanol	2 days	10.2	10.4
	2 days	10.1	10.5
Ethanol	None	11.2 <sup>c</sup>	10.2 <sup>c</sup>
	Dried at 100° C. in Abderhalden	None	11.2 <sup>c</sup>
	None	11.2 <sup>c</sup>	10.3 <sup>c</sup>

<sup>a</sup> All samples, freshly precipitated and humidified, dried at 70° C. in vacuum oven overnight previous to analysis. Humidification carried out by placing 2-gram samples of pectin, contained in crystallizing dishes having inside diameter of 45 mm., in large Pyrex desiccator containing 1 liter of water with a little toluene added.

<sup>b</sup> Sample of citrus pectin, 178 grade, dissolved in water to give concentration of 1%. Aliquots precipitated with indicated solvents.

<sup>c</sup> Calculated to moisture content after drying in vacuum oven at 70° C.

## DISCUSSION

Errors due to retained alcohol should be guarded against, particularly in studies of pectin structure, since most frequently pectin and its derivatives are precipitated from solution with ethanol, and ordinary drying technique does not remove all the alcohol. Pectinic acids prepared with pectinesterase showed the same tendency to retain ethanol. In this connection Morell, Baur, and Link (6) observed that citrus polygalacturonide, purified by extraction with 70% alcohol, still contained 0.4 to 0.6% methoxyl by the Zeisel method after treatment with alkali. Olsen *et al.* (7) obtained results in agreement with those of Link.

An attempt was made to use the de-esterification of pectin by alfalfa pectinesterase (pectase) as an analytical method for methoxyl determination in pectin. However, the enzyme method gave lower values than the Zeisel method on humidified pectins. In every case the enzyme hydrolyzed the pectins to a residual methoxyl content of 0.5%. Subsequent saponification accounted for most of the difference. Orange and tomato pectinesterases gave similar results, although the latter contained some pectinase. This extent of enzyme hydrolysis is not in accord with the findings of Hills, White, and Baker (3), who reported that tomato pectinesterase will hydrolyze pectin only to 1.8% of residual methoxyl, without describing their method for the determination of total methoxyl content of pectin.

The significance of the residual methoxyl not hydrolyzed by pectinesterase and a more detailed study of saponification will be reported at a later date.

## ACKNOWLEDGMENT

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# Automatic Distillation Apparatus for Gasoline Analysis

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An apparatus is described which makes possible the automatic operation of an efficient laboratory distillation unit. While the device was designed for gasoline analysis, it can be adapted to the control of other distillations. It automatically collects the fractions of distillate and plots the distillation curve. It has been operated 24 hours per day for well over a year with an operator present only 8 hours per day.

THE high level of the quality requirements specified for aviation gasoline makes it necessary to determine the composition of gasoline stocks in terms of individual hydrocarbons, in order to be able to produce the maximum amount of the desired components, and to eliminate or minimize production of materials of low quality. The first step in the analytical procedure is separation of the sample by distillation into fractions consisting of substantially pure hydrocarbons where possible, or, as a compromise, a mixture of a very few components.

Analytical distillation of gasoline requires a fractionating column with a large number of theoretical plates, and at the same time, small holdup per theoretical plate. In addition, as Rose (2, 3, 4) and his associates have shown, analytical distillations frequently require operation at reflux ratios of over 100 to 1, and use of a charge that is large compared to the holdup of the column in order to be able to isolate components present in small amounts. Under these conditions the distillation of a single sample may require several weeks of continuous operation. For this reason an automatic distillate-collecting device was constructed which makes possible the operation of a laboratory distillation unit with only occasional attention from the operator. This device uses some of the principles of the unit described by Bruun and Falconer (1) but is more nearly fully automatic. It was developed to be used with Podbielniak Super-Cal Model B distillation unit with a column containing 90 cm. (3 feet) of 22-mm. Heligrad packing, but it could, of course, be used with any type of laboratory distillation units.

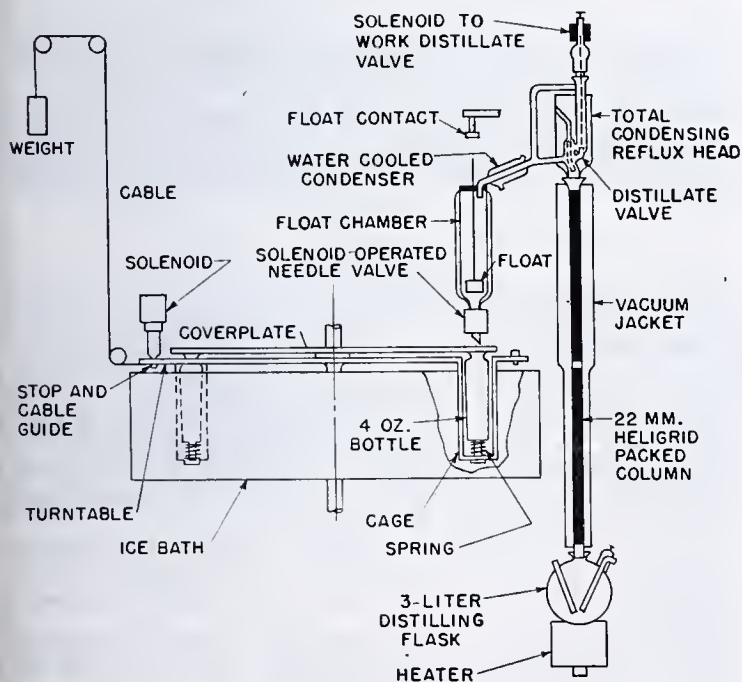


Figure 1. Sketch of Distillate-Collecting Device

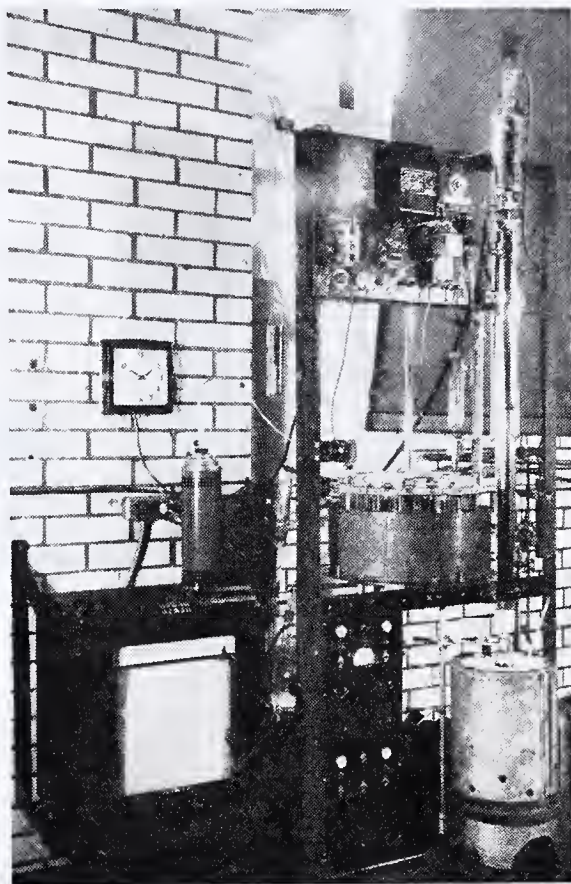


Figure 2. Automatic Distillation Apparatus

## DESCRIPTION

A sketch of the distillate-collecting device is given in Figure 1, and a photograph of the entire unit in Figure 2. The photograph was taken during the development of the apparatus and does not show several minor improvements.

As shown in Figure 1, the distillate from the total condensing reflux head passes through a cooler into a float chamber. When the desired amount of fraction has been collected in this chamber, the float strikes a contact, the solenoid-operated valve on the bottom of the float chamber opens, and the fraction drains into one of 18 bottles held in a turntable. The valve then closes and the turntable moves, bringing an empty bottle under the float chamber.

The float chamber is made of Pyrex and has a jacket through which cold water or other cooling medium can be circulated. The float is also glass and is sealed to a brass guide rod.

The solenoid-operated needle valve on the float chamber was made from an automotive carburetor needle valve (Figure 3), the seat of which was reground and the needle spring loaded to ensure a tight seal.

The fractions are collected in 120-ml. (4-ounce) oil sample bottles held in cages fastened to a turntable. Springs in the bottom of the cages press the bottles firmly against a cover plate. An effective seal between the bottle tops and the cover plate is obtained by using petrolatum as a lubricant on the cover plate. The cover plate is stationary and has a hole in it under the float chamber through which the bottles are filled. When the three bolts holding the plate are removed, it can be slid back and the bottles removed from the turntable.

Next to each of the 18 bottles and close to the edge of the turntable is a pin 1.25 cm. (0.5 inch) high and 0.6 cm. (0.25 inch) in diameter. One pin is always resting against the stop attached to the turntable solenoid (Figure 4). When this solenoid is energized the stop is raised and one pin allowed to pass under it.

The motive power for the turntable is supplied by a weight connected to the turntable by a cable through a system of pulleys.



The pins extend through the turntable and serve as cable guides on the underside.

An ice bath surrounds the hottles in the turntable. Ice is placed in a screened-off portion in the center of the bath, so that an ice jam cannot prevent the table from turning. The cover plate and turntable have holes through which the ice bath can be filled.

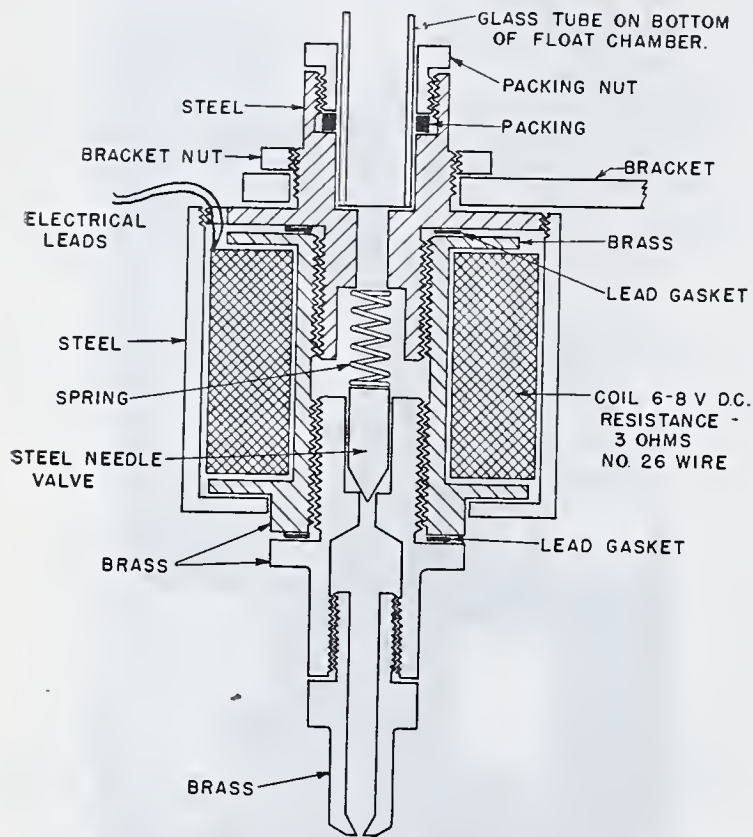


Figure 3. Cross Section of Solenoid-Operated Needle Valve

The only temperature recorder available was a potentiometer-type instrument covering the range of 0° to 400° F. with an iron constantan thermocouple. In order to improve the accuracy of the temperature measurement, a two-junction thermopile was used, thus doubling the sensitivity of the instrument for a given temperature change. The internal cold-junction compensator was balanced out by the use of a manganin resistor. For the low range of the instrument the external cold junction was placed in an ice bath, and for the high range in a steam bath. In this manner temperature measurements precise to  $\pm 0.6^\circ$  F. were obtained. The measurements must, of course, be corrected to a standard barometric pressure.

#### ELECTRICAL CIRCUIT

The electrical circuit as shown in Figure 5 has three different functions: (1) to control the collection of fractions, (2) to supply power to the recorder and cause the time at which a fraction is taken to be recorded, and (3) to stop the distillation when necessary.

When the float rises far enough to strike the adjustable float contact, a sensitive 6-volt direct current relay is energized. The closing of this relay causes a latch-in relay to close, and starts a vacuum tube timer. While the latch-in relay is closed, power is supplied to the float chamber needle valve, holding it open. After about 1 minute the vacuum tube timer releases the latch-in relay. This allows the needle valve to close and energizes the turntable solenoid just long enough to allow an empty hottle to move into position. A push-button is provided, so that this cycle can be started manually.

During the period that the latch-in relay is closed, a small 110-volt alternating current relay is also closed. The thermocouple leads are connected across this relay, so that when it is closed the thermocouple is shorted. This causes a break in the temperature record that shows when a fraction was hottled.

A circuit is provided to shut down the unit (1) when all 18 receiving bottles have been filled, (2) when the vapor tempera-

ture reaches some predetermined point, or (3) when the temperature goes off either end of the recorder scale.

As an alternative the unit can be made to go on total reflux. There are two alternating current circuits, one supplying the heater load and the other supplying the control load. The heart of the shutdown circuit is a sensitive direct current relay having one side of the alternating current control line across its contacts. This relay is energized from the battery booster when the reset switch is closed. There are four contacts, any of which when closed will short out this relay and thus break the alternating current control circuit. One contact is attached to the turntable, so that contact is made after the last bottle is filled. A contact is attached to each end of the recorder scale to prevent the temperature from going off the scale. The fourth contact is also in the recorder and can be set to make contact at any desired temperature, thus stopping the operation.

Since the contacts on the sensitive direct current relay are not large enough to carry the entire alternating current load, another relay is used to break the alternating current heater circuit. This relay is energized by the alternating current power from the control circuit. Thus, both safety relays are opened when any of the four safety contacts are closed. A switch is provided, so that the heater circuit may be kept on even if the alternating current control circuit is off. By the use of this switch the unit can be made either to go on total reflux or to shut down entirely when one of the four safety contacts is closed.

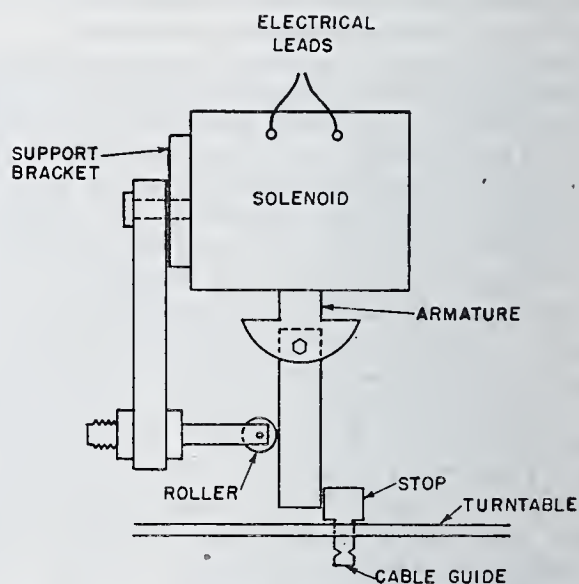


Figure 4. Turntable Solenoid Arrangement

For safety, 6-volt direct current power is used on all contacts. This prevents sparking when these contacts are closed and minimizes the danger of fire.

The alternating current control circuit includes the timer used to control the reflux ratio, which in this unit is an electronic timer having independently adjustable open and closed periods.

#### OPERATION

The distillation is run much the same as any other efficient laboratory distillation. A dehydrated sample is put in the distilling flask and the column brought to equilibrium at total reflux. The control circuit is turned on at the start of heating; however, the distillate valve solenoid is disconnected. The ice bath is filled and the float contact adjusted for the size of fraction desired. When equilibrium is reached, the collection of distillate is started by plugging in the distillate valve solenoid. This starting time is recorded by holding the thermocouple shorting relay closed for a few seconds.

Any pentane in the sample is distilled over while the operator is present, so that he may remove the fractions from the ice bath immediately. The pentane can be taken over rapidly, usually in 5 to 6 hours.

After the pentane has been distilled over, the heat input is adjusted to the desired boilup rate and the timer set for the desired reflux ratio. From this point until the end of the distillation the operator gives little attention to the unit. Every morning and evening he removes fractions from the turntable,



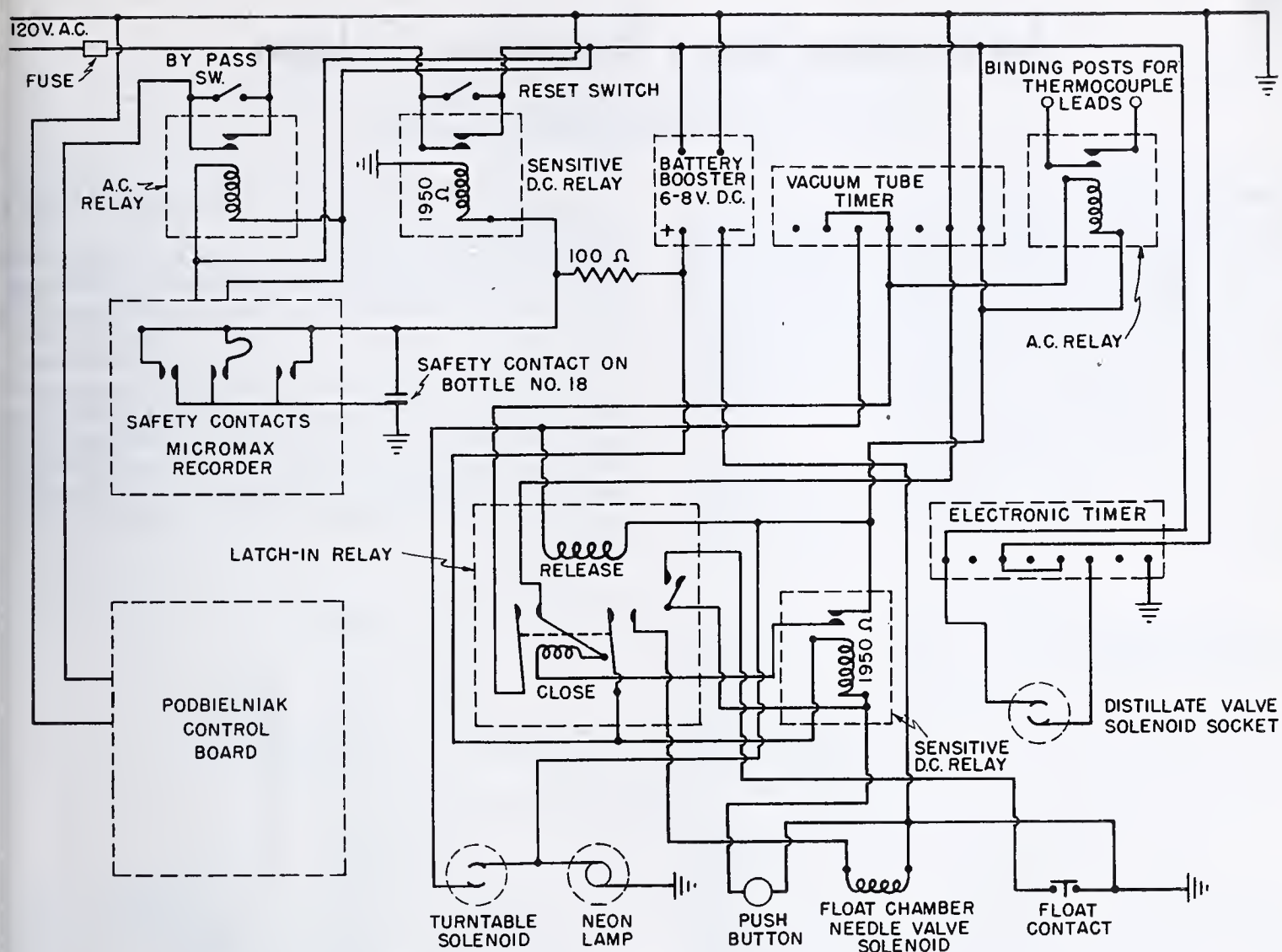


Figure 5. Wiring Diagram of Distillate-Collecting Device

winds up the weight, and checks the heat input to the column and the flask. Since less than 10% of the charge is normally taken overhead per day the heat inputs need only occasional adjustment. A variation of 5% in a reflux ratio of 100 to 1 has no appreciable effect on the efficiency of separation obtained. If, however, the unit were operated more rapidly or wide boiling range samples distilled, automatic control of the boilup rate would be necessary.

When the temperature approaches the end of the lower range of the recorder, the operator shifts the cold junction from the ice bath to the steam bath. Since there is a 25° F. overlap between the upper and lower scales, this change can usually be made sometime during the day. If the operator is not present, the unit shuts down when the end of the temperature scale is reached.

The size of fraction taken in this apparatus can be varied from 0.6 to 3% of the charge. Thus, small enough fractions can be taken to avoid overlapping of components and it is not necessary to be able to take cuts at given temperatures. If a number of cuts are obtained on a plateau, they can be blended to simplify the subsequent inspection work.

At the end of a run the recorder chart is removed and the distillation curve plotted. A section of a recorder chart showing the interruption in the distillation curve obtained when a fraction is bottled is shown in Figure 6. Since the chart speed of the recorder is fixed at 5 cm. (2 inches) per hour, the rate at which each fraction was distilled can be calculated from the size of cut and the length of chart covered by one fraction.

The fraction-collecting device described has been in use for over a year, during which it has been found extremely reliable. The presence of an operator is required for an 8-hour period at the start of a run, but thereafter for only an hour each morning and evening. Duplicate distillations check within the accuracy of the temperature recorder.

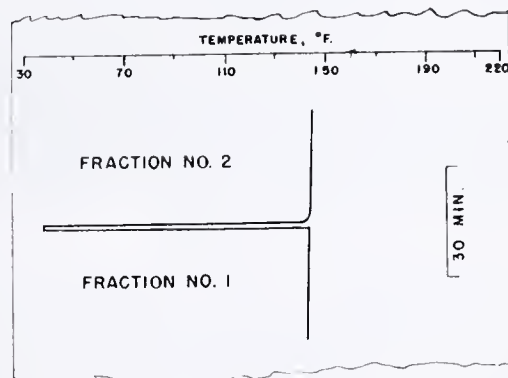


Figure 6. Section of Recorder Chart

#### ACKNOWLEDGMENT

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# Laboratory Spray Extraction Column

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THE laboratory extraction of many fluids of biological origin involves problems of emulsification and/or decomposition which the conventional apparatus cannot satisfactorily solve. When it is desired to extract the antibiotic principles from certain mold culture fluids, such as those of *Penicillium notatum* and *Aspergillus flavus*, this emulsification is a factor, but much more serious is the great lability of the penicillin and flavicin. To get good yields it is imperative to transfer these substances from the acidified culture fluids to the organic solvent in the least possible time and preferably at low temperatures.

In their early paper on penicillin Abraham *et al.* (1) mentioned the use of a spray column, but did not describe it. Shortly after that time the authors undertook to study penicillin, and were forced to develop this type of extraction apparatus, which is ideally suited to this problem.

In Figure 1 is illustrated the present arrangement, which was evolved from smaller columns. Of the factors not shown the following combination has been found satisfactory for extraction of penicillin or flavicin from culture fluid or from urine: pressure on the aqueous solution entering the jet head, 60 to 80 cm. of this solution; size of holes in jet head, 0.25 mm.; number of holes in jet head, about 40; rate of flow of organic solvent (isopropyl acetate), about half that of the aqueous solution. The throughput of the latter under these conditions is about 10 liters per hour if the jet holes are kept open.

The size of the jet holes is important. To obtain a maximum surface of the dispersed phase these holes should be as small as give satisfactory mechanical operation. Holes of 0.20-mm. diameter generally give a spray so fine that emulsification tends to be excessive, perhaps more important, they become plugged frequently by fine precipitates which are often present in the urine or culture fluid (they may be formed after filtration). With 0.25-mm. holes, on the other hand, emulsification is generally unimportant (even with culture fluid containing corn steep water) and the sediment usually passes through the holes readily. When the holes do become plugged they can usually be opened by a momentary increase of pressure.

The jet holes were made by a modification of the copper wire method of Branham and Sperling (2). In the flattened end of a Pyrex tube some 40 holes of about 0.4-mm. diameter were punched with a sharpened heated tungsten wire. These holes were arranged in the pattern of two concentric circles, the outer of 25 holes, the inner of 15 holes. Through each hole was placed a short piece of B. & S. No. 30 gage copper wire, bent at a right angle to hold it in place, and all the wires were fused into the glass by careful heating in an air-gas flame. After cooling the copper was dissolved out with nitric acid.

Most cultures of *Penicillium notatum* and most urines emulsify to some extent with the common organic solvents. Interference of emulsification with the proper functioning of the column can usually be reduced or eliminated by (1) removing the emulsion which accumulates below the solvent-inflow tube (the emulsion is withdrawn through the drain without interrupting the operation of the column by clamping the aqueous-overflow tube until the interface regains its proper position) or (2) choosing a satisfactory organic solvent. In the authors' experience isopropyl acetate is the solvent of choice. If the commercial product (95% pure) is washed with water, treated with anhydrous calcium chloride, and redistilled, the solvent gives a minimum of emulsification. Similar treatment of commercial *n*-butyl acetate (90%) gives a slightly less satisfactory solvent. Similar purification of various grades of amyl acetate gave a solvent which was distinctly less satisfactory than commercial *n*-butyl acetate.

Modifications of the spraying technique in which acetate was sprayed upward or ethylene dichloride was sprayed downward

through the aqueous solution gave emulsions which made operation impossible.

The solvent is chosen not only on the basis of these mechanical factors, but also according to the distribution of the desired solute. In the case of crude penicillin, for example, the distribution ratio between isopropyl acetate and water at pH 3 is about 15, whereas with isopropyl ether the ratio is much lower. If the distribution of the desired solute were not so highly in favor of the organic solvent, the column would have to be lengthened in order to get good yields with one pass. If the solute were sufficiently stable the aqueous overflow could be recirculated. However, it is possible to get some 80 to 85% of penicillin from culture fluid or urine into isopropyl acetate in one pass through the 1-meter column.

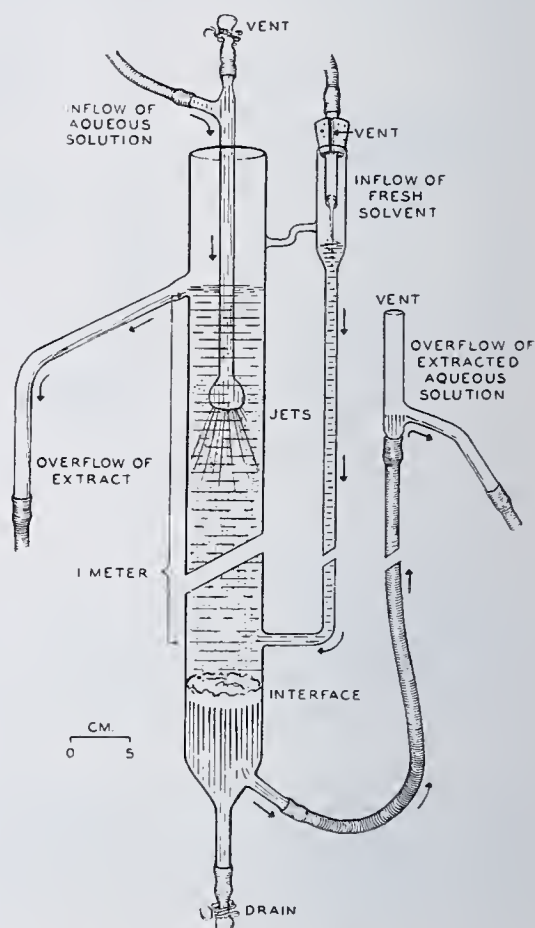


Figure 1. Spray Extraction Column

A batch of culture fluid or of urine containing penicillin or flavicin can be extracted as follows:

The fluid is poured through a fine "glass cotton" filter to remove coarse particles, then cooled to about 5° C. The column is filled with water and cold purified isopropyl acetate and the aqueous overflow tube adjusted so that the interface is just below the solvent-inflow tube. The culture is acidified in 400-cc. batches with phosphoric or hydrochloric acid to pH 3.0 ± 0.2, poured into a funnel some 60 to 80 cm. above the jet head, and allowed to spray into the acetate. Approximately 2 minutes are required for most of the 400 cc. to flow through. By acidifying and pouring in successive batches at intervals just often enough to maintain operation, decomposition of the penicillin is minimized. Fresh acetate is run countercurrentwise through the column at a rate

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of about 0.5 liter per liter of culture fluid. The acetate solution overflow is kept cold and is shaken out in batches with aqueous sodium bicarbonate. For 5 liters of acetate, three 50-cc. portions of about 0.3 *N* sodium bicarbonate are needed. The pH of each should be 7-8. These three bicarbonates are pooled and kept on dry ice pending purification. The amount of activity in this "crude extract" depends to a large extent upon the nature of the original fluid and of the active solute. From shallow cultures of *Penicillium notatum* containing 30 Oxford units per cc. or from urine having this activity or higher the yield is 80 to 85%. From shallow cultures of a strain of *Aspergillus flavus* containing only 1 to 2 Oxford units per cc. the yield is only about 50%. This high loss is principally due to decomposition in the acidified aqueous solution.

The mechanical mixing of the culture fluid and acid in the proper proportions has been considered, and it appears that suitable equipment is available for the purpose (from Wilson Pulsafeeders, Inc., 205 Clinton St., Buffalo 5, N. Y.). Much smaller amounts of culture could thus be acidified at more frequent intervals, reducing losses by decomposition. A Pulsafeeder could also be made to pump the fresh acetate, and possibly also to pump the acetate solution upward continuously through a bicarbonate column, or perhaps better spray the bicarbonate downward through the acetate flowing upwards in a column.

Possibly on a larger scale these continuous operations could be carried out more efficiently than is feasible on a small laboratory scale.

#### SUMMARY

A laboratory spray extraction column is particularly useful for extracting aqueous fluids of biological origin with solvents lighter than water. Advantages over the more common laboratory extraction apparatus are that emulsification interference can usually be entirely circumvented, and transfer of solute to the organic solvent takes place in a minimum of time. It offers these possible disadvantages: (1) relatively large volumes of solvent are needed; (2) the distribution of the desired solute must be favorable.

In recovering penicillin or flavicin from culture or urine these disadvantages are unimportant compared with the advantages.

#### ACKNOWLEDGMENT

Funds for this work were kindly given by the Mallinckrodt Chemical Works.

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## A Fractionating Molecular Still

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A molecular still is described in which fractionation is effected through multiple distillation. A series of communicating stages of distilling and condensing surfaces, in which material of greater distillability is progressively advanced to forward stages and material of lesser distillability refluxed to rearward stages, is provided in a single unit.

IN THE course of a program instituted in this laboratory to isolate and characterize the active principle (or principles) of marihuana, *Cannabis sativa L.* (11), it was proposed to separate the components of the resin, in which the physiologically active material is contained, by fractional molecular distillation. This operation was performed initially in a pot-type molecular still, similar to that described by Mair, Schicktzan, and Rose (9). In this type of still, material which is evaporated from the surface of the distilland condenses on the roof, flows into an annular gutter which prevents its return to the distilland, and is conducted directly to receivers.

Two major factors limit the extent to which fractionation can result from molecular distillation in a still of this type. One results from the lack of provision for constant renewal of the distilling surface, such as is provided by ebullition in ordinary distillation. Diffusion of molecules to the surface layer is retarded by the usually viscous nature of the distilland. Molecules of greater volatility, which normally would preferentially distill from the surface, are thus entrapped in the body of the distilland, and the distilling surface becomes correspondingly enriched in molecules of lesser volatility. Since only molecules evaporated from the surface of the distilland reach the condenser, the extent to which a representative portion of the whole distilland is subjected to distillation is seriously affected. Redesign of the molecular still has been necessary to circumvent this limitation. The so-called "falling-film" type of still, in which the distilland flows by gravity over a vertical heating surface, was first described by Hickman (8); a number of other stills employing this prin-

ciple have also been designed (7, 10, etc.). In these stills, the combined effect of thin layer of distilland and constant movement of the distilland results in a material improvement in the extent to which the distilland surface will be representative of the entire charge.

The second major factor limiting the extent to which fractionation can take place in the pot-type still is the absence of means for multiple distillation. In molecular distillation, as in distillations conducted at higher pressures, the degree of separation is a function of the respective rates of evaporation of the various molecular species. The ratio of the components of a mixture in the distillate from any single distillation is proportional to the partial pressures of the components in the distilland; in molecular distillation it is also inversely proportional to the square roots of their molecular weights.

In order to improve the degree of separation in ordinary (equilibrium) distillations, fractionating columns are used which provide, in effect, successive stages of distillation, the number of effective redistillations being expressed in terms of theoretical plates. In molecular distillation, however, the type of fractionating column dependent upon the establishment of equilibrium between liquid and vapor obviously cannot be employed. In the falling-film type of molecular still, fractionation is achieved through passage of the distilland over the heating surface maintained at a definite temperature; the maximum theoretical fractionation which can result in this way is equivalent to one perfect plate. To attain further fractionation, complex arrangements of combinations of still units designed to simulate the performance of fractionating columns have been employed (4, 5, 6).

The present communication describes a simple apparatus which provides for multiple distillation, while retaining the conditions necessary for molecular distillation. This is accomplished by providing within a single unit a series of communicating stages of distilling and condensing surfaces. As in the simple still, distillate from an initial stage is condensed on the roof of the still and engaged by a trough during its downward flow. In-

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stead of being removed from the still, however, the distillate is directed by the troughs to a succeeding heating surface, located at a higher level than the preceding one. Here the more readily distilled portions are again preferentially evaporated, and again carried to the next stage. While the more volatile material progressively advances, the less volatile material continuously regresses on the floor of the still toward rearward heating surfaces. By applying heat in graduated amounts to the several sections of the still, with the greater amounts applied at the initial stages, the desired rate of distillation and ratio of reflux may be maintained.

The heating areas are defined by means of transverse ridges on the floor of the still, which act as dams checking the rearward flow of liquid and causing it to form shallow pools. The troughs are so located in relation to the pools as to engage distillate from each pool and conduct it to the succeeding pool. Liquid in each area is constantly replenished both by distillate from preceding areas, conveyed by the troughs, and by reflux from the superiorly located areas. The constant rearward flow of liquid along the heating surface and the merging of the counterflowing distillate and distilland at each stage serve to agitate and continuously renew the surface of the distilland.

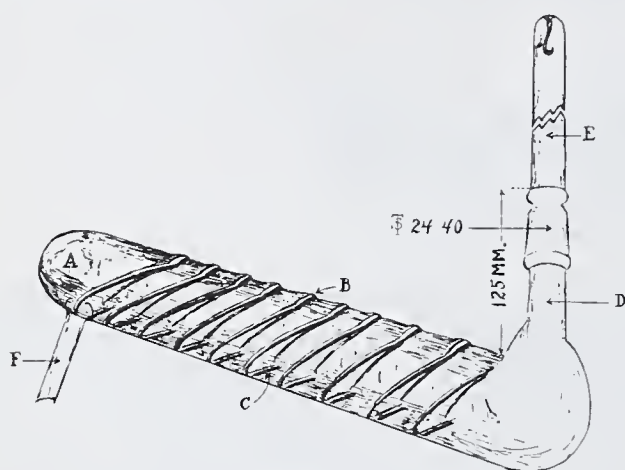


Figure 1

The several heating areas are not distinct; a continuous film of liquid covers the entire floor of the still, thereby providing the large distillation surface which is important in molecular distillation. The condensing areas on the roof of the still are, however, essentially distinct, each stage being the area between successive troughs. Material evaporated from any point on the heating surface is condensed on the surface immediately above it, and is necessarily carried to a forward point for redistillation.

The uppermost trough leads directly to a take-off tube, through which the distillate is conveyed to the receiver system. The still is connected to the usual high-vacuum system, in which operating pressures of  $10^{-4}$  mm. of mercury or less are maintained.

#### DESCRIPTION OF APPARATUS

The authors' still (Figures 1 and 2) is constructed of a cylindrical Pyrex tube, A, 38 cm. long and 50 mm. in diameter. The interior surface of the tube is provided with a series of 10 channels, B, approximately 3 mm. deep, placed at an angle of  $45^\circ$  with the axis of the tube and 25 mm. apart. These extend around the tube except for a space of 20 mm. at the bottom, where lateral ridges, C, 2 mm. high are situated 5 mm. behind the termini of the channels. A vertical tube, D, 22 mm. in diameter and 125 mm. in length, and provided with a male  $\frac{1}{2}$  24/40 joint, is sealed onto the top of the tube, at the lower end. The cap, E, of this opening is 30 cm. in length. A thermometer is suspended on a glass hook sealed onto the top of the cap, reaching to within several millimeters of the floor of the still. An outlet tube, F, 12 mm. in diameter is sealed onto the bottom of the tube, at the upper end. This leads directly to the vacuum and receiver system. The termini of the uppermost internal channel lead directly into this tube.

Heat is provided by a Nichrome wire heater, built in four

sections. Each section consists of four parallel coils, separated by mica strips, connected in series. The individual coils are approximately 75 mm. in length and 4 mm. in diameter, and are made by wrapping 60 cm. (2 feet) of No. 22 B. & S. Nichrome wire around a rod. Each heater unit is connected through a common ammeter to an individual switch and 3.5-ampere rheostat; the current passing through each unit can be read separately on the ammeter. The heater is fastened to an asbestos-board floor of a metal box,  $36 \times 7.5 \times 5$  cm., one end of which is partially cut out to adapt it to the still. The still is seated on asbestos paper covering the heating coils, and is cemented in the box with magnesia-asbestos plaster. The box

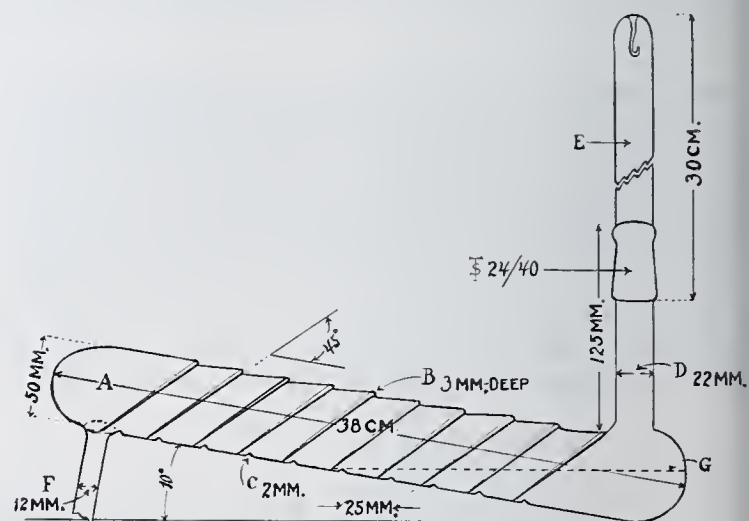


Figure 2

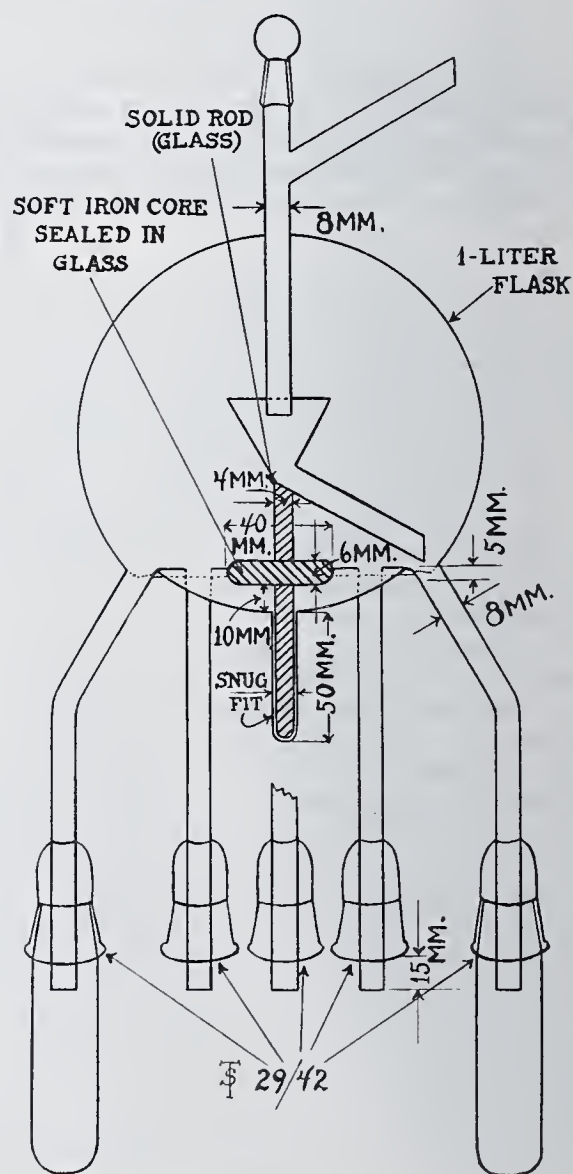


Figure 3



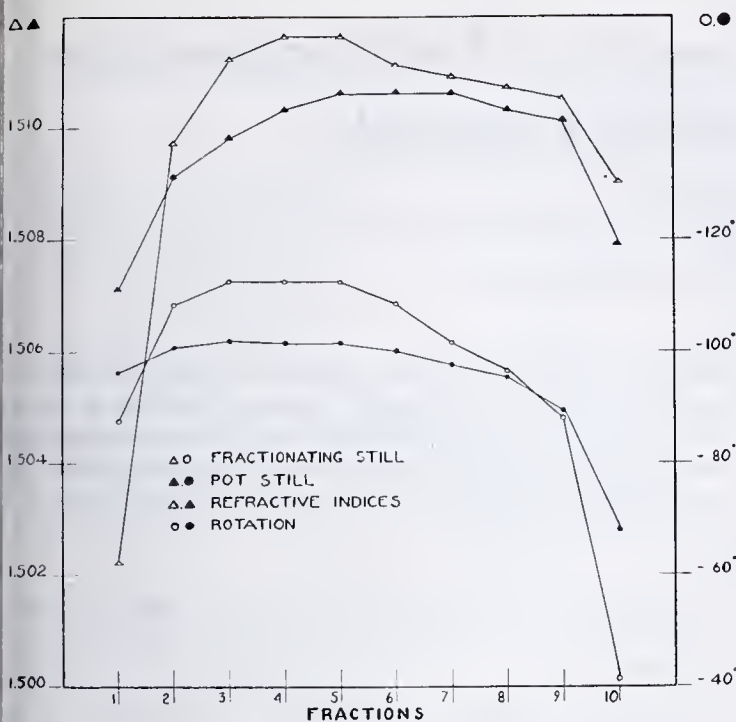


Figure 4

is clamped on a rigid frame, in such a position as to fix the still at an angle of  $10^\circ$  with the horizontal.

The outlet tube of the still is connected directly to a vacuum line and to the receiver system, in which fractions may be collected in individual receivers without interruption of vacuum. This apparatus (Figure 3) is a modification of the Brühl apparatus (12). (The apparatus used in this laboratory was designed by S. T. Schicktanz.) It consists of a central chamber to which are attached ten radial arms, each bearing a  $\frac{1}{8}$  29/42 joint. Distillate is directed to the arms through a funnel, the stem of which is bent at an angle as shown in the drawing. The funnel is mounted on a vertical glass rod, which serves as a pivot and bearing. A soft iron rod is sealed horizontally into the mounting of the funnel; an external electromagnet is used to turn the funnel, thus directing the stem toward the desired receiver arm. Distillate is collected in test tubes resting in the caps which are used as closures of the arms.

The vacuum system, consisting of a 1-liter ballast flask, dry ice trap, McLeod gage, and mercury vapor diffusion pump, backed by a fore-pump, is assembled in the usual manner.

#### OPERATION

In operation, the still is charged through opening *D* to the level indicated by the broken line, *G*, and the system is closed. The first heater unit is turned on low, and the charge is degassed by gradual reduction of pressure. During the degassing operation a small amount of material may be spattered on the roof of the still, especially if the pressure is reduced too rapidly; this will be washed down by the first portions of distillate during the actual distillation.

After the charge has been thoroughly degassed, the diffusion pump is started and a greater amount of heat applied to the first heater unit until distillation proceeds. Material evaporated from the surface of the main charge will condense on the roof of the still and flow laterally around the inner surface of the still until engaged by a channel, *B*, along which it will flow downward and forward to a succeeding heating area. As distillation progresses, the condensate forms pools in these areas and overflows, forming a continuous flowing film on the floor of the still. The succeeding heating units are cut in as the areas they govern become supplied with distilland. Distillation from each area proceeds in the manner described, and the more volatile components are progressively carried forward while the less volatile components flow in a rearward direction. The rate of distillation and reflux is governed by the heat input at the several heater sections; by the application of a proper differential in the input of the succeeding sections, as indicated on the ammeter, a proper rate of reflux may be maintained.

Distillate at the uppermost stage is conducted by the final trough in the series to the outlet tube and thence to the receiver system, where it is directed to the desired individual receiver by manipulation of the funnel. Fractions may be cut on a basis of equal volume or in accord with an obvious change in some physical property, such as color or viscosity.

The extent of the improvement in fractionation attained with the fractionating still as compared with the pot-type still is illustrated in Figure 4.

Portions of an acetylated marihuana "red oil", twice previously distilled to remove unlike components, were distilled in each type of still at a pressure (McLeod gage) of  $10^{-4}$  mm. Ten fractions of approximately 8 grams each were collected. The specific rotations and refractive indices shown indicate a significant differential between the two series of fractions. An evaluation of the significance of this differential may be made by comparison with the results obtained in the distillation of marihuana red oil by other workers. Bergel (1), for example, construed failure to obtain differences in the physical constants of the several fractions resulting from ordinary high-vacuum distillation as evidence that the red oil was an individual compound.

#### DISCUSSION

The apparatus as described may readily be adapted to considerable variation, while retaining the features by virtue of which fractionation results. By appropriate modification, for example, distilland could be introduced in a continuous fashion at some intermediate point in the still, rather than being placed in the still initially as a single charge. The charge could be provided from a reservoir of predegassed material; or distillate from a "falling-film" type of apparatus could be conducted directly to a point on the heating surface perhaps one third of the distance from the initial stage. Apparatus constructed in this manner would be especially desirable for the distillation of thermally labile material.

During the distillation of marihuana oils, the McLeod gage regularly indicated a residual gas pressure of less than  $10^{-4}$  mm. of mercury. It is recognized that this does not indicate the actual distillation pressure; it does, however, indicate air-tightness of the system and absence of decomposition of the charge to gaseous products. Considerations of the magnitude of the actual distillation pressure must not, in any event, be made in accord with the ordinary concepts of pressure. The flow of evaporated molecules during molecular distillation is essentially unidirectional, while the usual concepts of pressure relate to random movement of molecules. During unidirectional distillation, the mean free path of the molecules is, as pointed out by Brønsted and Hevesy (2), much greater than the mean free path under ordinary conditions. Indeed, the interrelation between direction of movement and magnitude of mean free path is incorporated in the definition of mean free path which was proposed by Langmuir (3). There may be, then, considerable latitude in the dimensions of the still and in the rate of distillation in so far as they relate to adherence to the principles of molecular distillation.

#### ACKNOWLEDGMENT

The authors wish to thank S. T. Schicktanz for furnishing the design of the receiver, and Edith J. Poindexter for preparation of the drawings.

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APPLICATION has been made for patent on this still.



# Apparatus for Automatic Control of Electrodeposition with Graded Cathode Potential

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AND  
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An apparatus is described for carrying out graded cathode potential electrodepositions automatically, whereby a metal may be separated by cathodic deposition from a metal lying closely above it in the electromotive series. The device consists of a vacuum tube amplifier which magnifies the cathode-calomel voltage sufficiently to actuate a relay and motor which drives a Variac; the Variac governs

the size of an alternating current, which when rectified is used to effect the deposition. The entirely automatic operation of the apparatus frees the analyst for the entire period of the electrodeposition and shortens the time normally taken for such an analysis. The apparatus has been tested on the separation of copper from tin in hydrochloric acid solution.

IN THE normal practice of analysis by the electrodeposition of a metal at the cathode, the voltage necessary to yield a current of convenient size is applied initially to the cathode and anode and no further attention paid to it other than to change its value occasionally to maintain the current as the composition of the electrolyte changes. By such a constant current electrodeposition the possible separations are limited to those metals below hydrogen in the electromotive series from those above hydrogen, hydrogen being evolved after the deposition of the lower metal in preference to deposition of the higher metal. The change in the cathode-anode voltage during the electrolysis is no clue to the extent of the deposition of a metallic ion, but is the algebraic difference of the voltages between the solution and the cathode and anode and the  $IR$  drop through the solution, all of which may undergo change during the electrolysis.

By inserting a reference half-cell into the solution and measuring the voltage between the cathode and the reference cell it becomes possible to isolate the effect at the cathode. The voltage between the solution and the cathode consists of the equilibrium voltage of the electrode metal toward the solution containing its ions and concentration polarization caused by the flow of current. Neglecting the latter for the time being, there is thus provided a means of following the change in the concentration of the metal ion during the deposition, the reversible voltage being given by the Nernst equation:

$$E = E_0 + \frac{RT}{nF} \ln [M^{n+}]$$

Thus, for example, to separate copper from tin, the apparatus shown in Figure 1 is employed, a calomel half-cell being used as the reference electrode. The cathode-calomel voltage, measured by the potentiometer, increases as the copper is deposited. Using a 0.1 *N* calomel reference half-cell, this voltage is not allowed to exceed 0.45 volt, the electrolyzing current being decreased progressively by increasing the resistance,  $R$ , to accomplish this. The last copper will be gradually deposited by the successively smaller current without the deposition of any tin.

Since their first use by Sand (3) such graded cathode potential separations have not become popular, even though a number of very useful applications of the method have been devised, notably by the English workers Sand, Lindsey, Collin, and Torrance. The continuous attention of the analyst is required throughout the electrolysis and the deposition can seldom be completed in less than 60 minutes, since it is necessary to use a relatively low initial current, less than 2 amperes. At greater currents the changes of the cathode voltage occur so rapidly that the manual operations of balancing the potentiometer and adjusting the current cannot be carried out sufficiently rapidly. Clearly then, this is a case for automatic control.

The apparatus described, which accomplishes the graded cathode potential separation automatically, consists of three main units:

1. A rectifying unit by which a direct current output of low voltage can be obtained from the 110-volt alternating current line to perform the electrolysis.
2. A control circuit consisting of a vacuum tube amplifier, a relay, and a motor-driven Variac by which the cathode voltage is made to govern the electrolyzing current.
3. A vacuum tube voltmeter for convenience in measuring the cathode-calomel voltage.

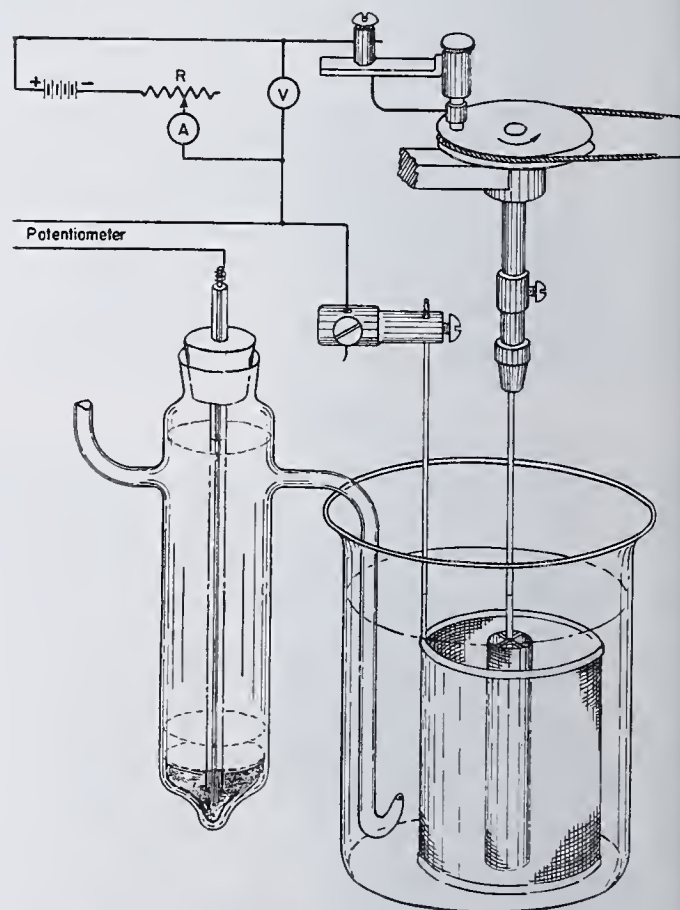


Figure 1. Circuit and Apparatus for Graded Cathode Potential Electrodeposition

The circuit and appearance of the apparatus are shown in Figures 2 and 3.

## RECTIFYING CIRCUIT

The direct current needed for the electrolysis is obtained from the 110-volt alternating current line, the circuit elements being, successively, a switch in the 110-volt alternating current input, a Variac, a step-down transformer, a selenium rectifier, a milliammeter-shunt combination, and a filter.



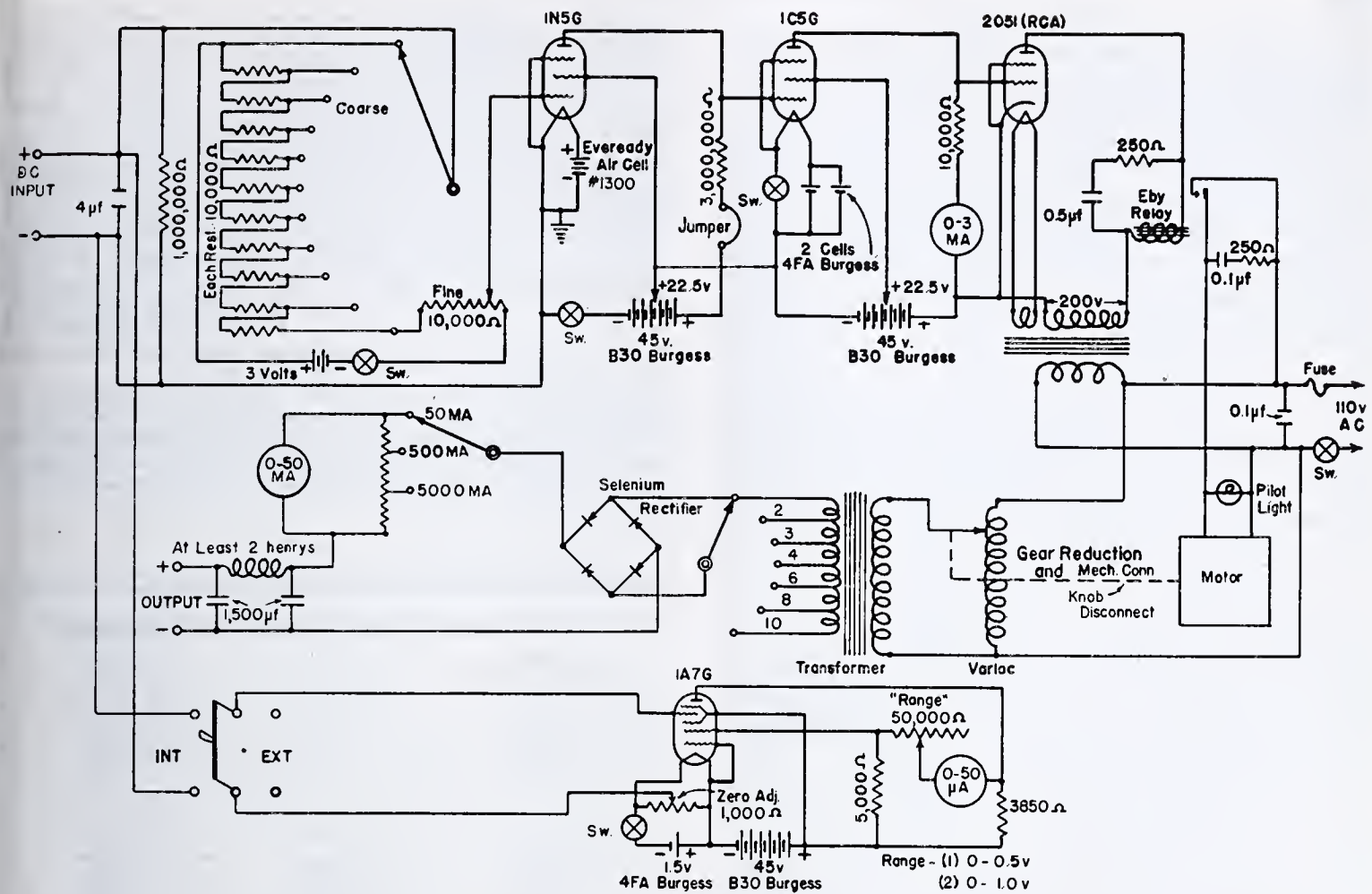


Figure 2. Circuit of Apparatus for Automatic Graded Cathode Potential Electrodeposition

Relay H. H. Eby Co., Philadelphia, Type ER12, 10,000 ohms. Variac, General Radio Co., Cambridge, Mass., Type 200B. Motor, 1/50 h.p. induction motor with 1200 to 1800 r.p.m., gear reduction to turn Variac about 0.5 r.p.m. Rectifier, I.T. and T. selenium rectifier, Type 4B1C4

The Variac is driven by a motor activated in turn by the amplifier-relay (control) circuit and is the mechanism whereby the electrolyzing current is decreased as a result of increases in the cathode-calomel voltage. The Variac may be set by hand after turning a knob which disengages it from the motor. The secondary of the step-down transformer has taps to provide voltages of 2, 3, 4, 6, 8, and 10 volts when 110 volts are supplied to the primary of the transformer by the Variac. Thus, no more than a safe load can be supplied the selenium rectifier which is capable of handling up to 10 volts.

The rectifier is followed by a filter consisting of an inductance of 2 henrys in series and two capacitances of 1500  $\mu$ f in parallel, one before and one after the choke. The purpose of the filter is to smooth out the 120-cycle ripple which otherwise affects the control circuit adversely. The direct current drawn is measured by a milliammeter suitably arranged with three shunts, so that it can be made to read three current ranges, 0 to 0.05 ampere, 0 to 0.5 ampere, and 0 to 5.0 amperes, by changing the position of the range selector knob on the lower right front panel of the instrument.

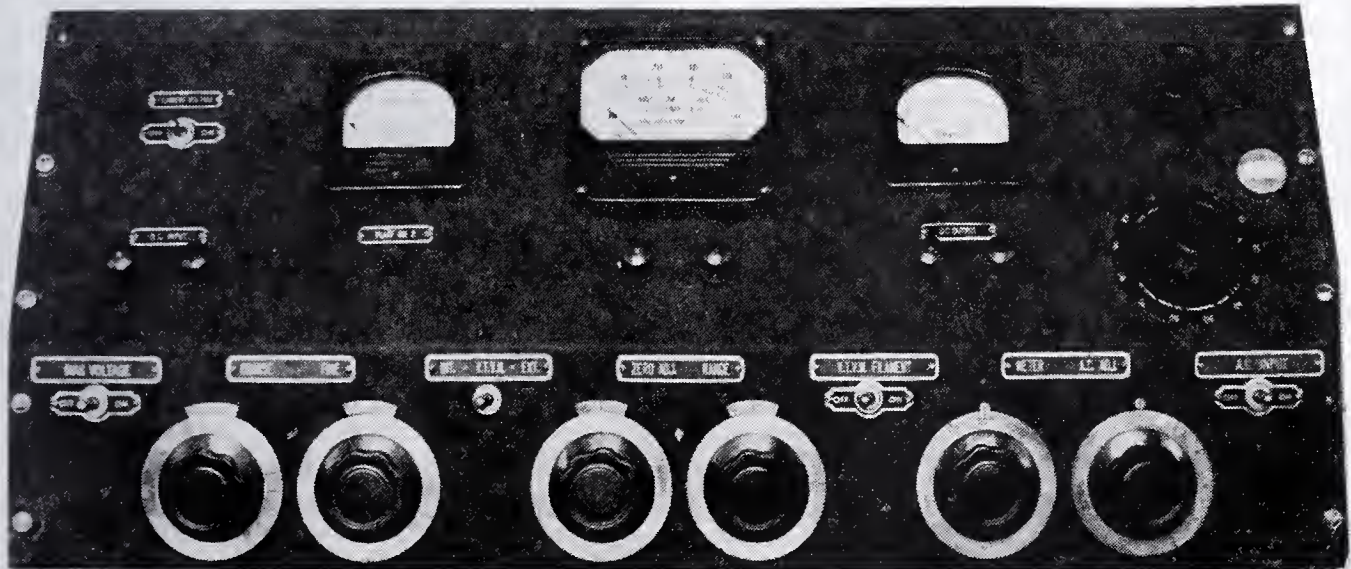


Figure 3. Apparatus for Automatic Graded Cathode Potential Electrodeposition



## CONTROL CIRCUIT

The cathode-calomel voltage is amplified by a two-stage vacuum tube amplifier which drives a gas-filled tube which provides enough power to operate a relay. The relay in turn controls a motor which turns the Variac.

It was desired to have a control circuit which would respond to a change of 10 millivolts in the cathode-calomel voltage. Numerous attempts were made to design a line-operated amplifier of sufficient sensitivity. It appeared impossible to stabilize the filament supply when operated from the alternating current line voltage and it was necessary to resort to battery operation. (Numerous attempts to design a reasonably simple, line-operated amplifier are discussed by Parker, 2.) The vacuum tubes selected were chosen from the low filament current tubes recently made available; power required is so low that the batteries last over 6 months in continuous operation.

The cathode-calomel voltage is applied so as to buck the grid bias of the first tube, a 1N5G; as the cathode-calomel voltage increases the cathode-grid voltage of the 1N5G becomes less negative, allowing more current to flow in its plate circuit. This increase of the plate current causes the grid of the second tube, a 1C5G, to become more negative and the plate current of this tube decreases, in turn causing the grid voltage of the third tube, a gas-filled tube, Type 2051, to become more positive. If the grid voltage of this tube becomes more positive (less) than -2.0 volts, the critical value for firing at the plate voltage used, it passes current sufficient to close the relay.

The grid bias voltage of the first tube is secured from a 3-volt battery with a potential divider with coarse and fine adjustments. The total resistance of the potential divider is 110,000 ohms in steps of 10,000 ohms, the coarse settings being fixed 10,000-ohm resistors and the fine adjustment a variable 10,000-resistor. The coarse setting thus changes in steps of about 0.27 volt and the fine adjustment permits setting to about 0.005 volt.

The cathode-calomel voltage (C.C.P.), the bias voltage (B.P.), and the grid voltage (G.P.), which is just sufficient to activate the relay, are related by:  $B.P. - C.C.P. = G.P.$  Thus for any calomel-cathode voltage there is a corresponding bias voltage which causes the relay to close. The bias setting may be calibrated in terms of the input voltage which just closes the relay, by impressing a known voltage on the input terminals and adjusting the bias until the relay just closes. The calibration is essentially linear and usually holds for about 2 weeks, after which the unit must be recalibrated. The use of the vacuum tube voltmeter makes such readjustments a simple matter.

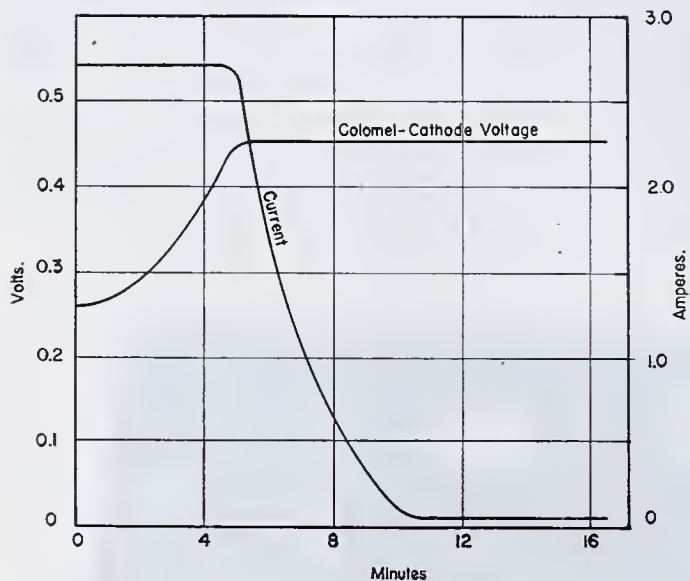


Figure 4. Course of Cathode-Calomel Voltage and Electrolyzing Current during Deposition of Copper in Presence of Tin

In the separation of copper from tin, the calomel-cathode voltage (0.1 N calomel) must be limited to 0.45 volt. This corresponds to a certain bias setting—for example, coarse reading 8, fine 27.0. With the bias so adjusted any voltage greater than 0.45 volt applied to the D.C. input causes the relay to close. The electrolyzing current is then reduced until the calomel-cathode voltage falls below 0.45 volt, at which point the relay opens.

The 2051 tube which drives the relay is a gas-filled tetrode which passes a comparatively high current, about 9 milliamperes, once its grid voltage becomes less than -2.0 volts. Once the plate current has started, its magnitude is determined by the anode supply voltage and the impedance of the anode circuit and is practically independent of the grid voltage. By operating the 2051 on alternating current it is possible to have the tube turn off when the grid voltage becomes only slightly more negative than -2.0 volts. During the negative half cycle of the alternating current voltage applied to the plate, the plate current is zero, since the plate is negative with respect to the cathode. As long as the grid voltage is less negative than the critical value of -2.0 volts, the tube will conduct on positive half cycles. However, if the grid is more negative than the critical value, conduction will be prevented on positive half cycles. In other words, a change in grid potential can cause the 2051 tube not only to close but to open the relay.

A milliammeter (left-hand meter, Figure 3) was placed in the plate circuit of the second tube as an indication of approach to the point where the relay closes. This is convenient in showing that the amplifier is functioning and is a somewhat more sensitive indicator than the vacuum tube voltmeter of the changes in the cathode-calomel voltage.

Table I. Standardization of Copper and Tin Solutions

Copper Solution Taken	Copper Found	Concentration of Copper
Grams	Grams	G./g. of solution
36.313	0.7560	0.020819
48.194	1.0033	0.020819
48.747	1.0146	0.020814
		Av 0.020818
Tin Solution Taken	Tin Found	Concentration of Tin
21.982	0.4243	0.019278
29.547	0.5701	0.019295
30.706	0.5913	0.019261
30.405	0.5852	0.019246
38.065	0.7335	0.019270
		Av. 0.019270

## VACUUM TUBE VOLTMETER

For convenience in measuring the cathode-calomel voltage, a vacuum tube voltmeter was incorporated in the apparatus. The recent design of Garman and Droz (1) was used, employing a single, battery-operated tube and making use of a bridge-type circuit. Because of the low power consumption (0.3 watt) the inconvenience and cost of frequent battery replacement are reduced to a minimum and the added advantage is secured of greater stability and greater simplicity than with line operation. The circuit is shown in Figure 2.

The vacuum tube voltmeter was placed in the center of the apparatus and shielded by enclosure in a separate metal box. The zero and range adjustment controls, meter, and filament switch were brought to the center of the front panel, as will be seen from a close inspection of Figure 3. In order to use the vacuum tube voltmeter for other purposes if desired, input terminals were placed on the panel (just below the meter) and a switch provided so that the voltmeter could be made to read the cathode-calomel voltage (int.) by direct connection within the apparatus with the D.C. input, or to read some other voltage applied to the voltmeter input terminals (ext.).

The vacuum tube voltmeter is calibrated by first shorting the input terminals and varying the zero adjustment to bring the meter to read zero. The desired voltage to cause full-scale reading is then applied and the range adjusted until the meter reads full scale. The zero is then checked and adjustment made if necessary. The full-scale setting is then checked and adjustment made if needed. The voltmeter may be calibrated over any range up to 1 volt—for example, 0 to 0.5 volt or 0 to 1.0 volt.

## AUXILIARY EQUIPMENT

In graded cathode potential electrodepositions, vigorous stirring is essential. This is most conveniently accomplished with a rotating platinum anode driven at about 800 r.p.m. It was found that electrical contact to the rotating anode could be made conveniently by a carbon brush bearing on the flat upper surface



of the pulley attached to the chuck holding the electrode; some minor variation of a few milliamperes in the electrolyzing current occurred because of the unevenness of this contact, but this had no effect on the operation of the apparatus.

The conventional form of calomel electrode may be used, 0.1 N, 1 N, or saturated, the appropriate change in the limited potential used being made on substituting one for another. The calomel cell must be placed with its contact tip on the outer side of the cathode and the tip should not extend below the lower edge of the cathode, so as to be as far away from the lines of flow of electrical current as possible.

The electrode assembly should be designed so that the beaker can be lowered away and the electrodes washed quickly following the electrolysis.

OPERATION

The filament voltage, bias voltage, and vacuum tube voltmeter filament are turned on and the apparatus is given a 20-minute warming up period before starting the electrodeposition. Connections from the D.C. output are made to the cathode and anode and from the D.C. input to the cathode and calomel cell. In the case of copper and metals higher than copper in the electromotive series, the calomel cell is connected to the positive terminal. The bias controls are set for the limited potential wanted. The stirring motor is started and the electrolysis is begun by turning the alternating current switch on, setting the Variac to full value, and turning the voltage regulator to give a suitable current.

It is best then to test the D.C. input circuit by breaking contact at the calomel cell junction. The reading of the vacuum tube voltmeter should change on breaking the contact or upon altering the size of the electrolyzing current. If a variation is not observed, the other contacts should be examined and the calomel cell inspected for air bubbles. If the circuit is closed the electrolysis can proceed without further attention from the operator.

The electrolysis is usually complete in 20 to 40 minutes, by which time the current will have been reduced to 20 milliamperes or less. In some cases the current must not be allowed to fall below a certain value, below which the metal may dissolve more rapidly than it is being plated out. The solution is then removed, the electrodes washed without interrupting the current, and the determination concluded in the usual manner.

Pure tin was dissolved in hot, concentrated hydrochloric acid in contact with metallic platinum, a reflux condenser being employed to prevent the loss of tin by volatilization. The solution was diluted carefully and the tin determined electrolytically. The solution was measured out with a weight buret, diluted to 250 ml., and treated with 10 grams of hydroxylammonium chloride. The tin was then deposited on a copper-plated platinum electrode, using a current of 1.5 amperes. Just before discontinuing the electrolysis the solution was neutralized with ammonia to avoid any solvent action of hydrochloric acid on the tin during the washing process. Following the deposition the solution was checked for the complete removal of tin. The results are given in Table I. This solution contained about 5 ml. of free, concentrated hydrochloric acid per 25 ml. It was found that if the solution was not diluted to about 250 ml. the tin deposited in part as large crystals at the top and bottom of the electrode.

Quantities of these solutions, delivered from a weight buret, were mixed and to the resulting solution were added 10 ml. of concentrated hydrochloric acid and 2 grams of hydroxylammonium chloride. The solution was then diluted to 150 ml. and the electrolysis begun at a current of 2 to 4 amperes. The apparatus was set to limit the cathode-calomel voltage to 0.45 volt (0.1 N calomel). The electrolysis was allowed to continue until the current had been decreased to about 0.020 ampere.

The course of the current and the cathode-calomel voltage during a typical deposition of copper are shown in Figure 4, the quantities of copper and tin present in the particular determination from which the data was obtained being, respectively, 0.3222 and 0.5190 gram. The action of the apparatus is clearly apparent. Once the critical potential, as set by the bias controls, is reached the calomel-cathode voltage is held constant and the current decreased to a residual value approaching zero. The electrolysis was generally continued until the residual current was about 20 milliamperes, the time required being from 20 to 40 minutes, depending on the amount of copper present.

Following the deposition of copper, about 8 grams of hydroxylammonium chloride were added, the solution was diluted to about 250 ml., and the tin deposited on a copper-plated platinum cathode.

The results of a series of separations of copper from tin are given in Table II. The separation is very successful as long as a large amount of tin is present, as in the analyses reported in Table II. When the amount of tin is less than that equivalent to the copper present the separation becomes erratic, low results being frequently obtained for copper. This peculiar behavior resides in the electrochemistry involved, however, and not in the action of the apparatus described, since similar erratic results were obtained on low-tin mixtures by the conventional, manual method of carrying out the graded cathode potential electrodeposition. Additional tin can be added when necessary, as, for example, in the direct determination of copper in bronze.

Table II. Separation and Determination of Copper and Tin

Copper Taken				Tin Taken			
Solution Grams	Copper content Gram	Copper		Solution Grams	Tin content Gram	Tin	
		Found Gram	Error Mg.			Found Gram	Error Mg.
13.819	0.2877	0.2876	-0.1	25.5	0.48	Not determined	
22.700	0.4726	0.4721	-0.5	25.5	0.48	Not determined	
22.902	0.4768	0.4763	-0.5	25.5	0.48	Not determined	
6.332	0.1318	0.1318	0.0	42.420	0.8175	0.8169	-0.6
12.701	0.2644	0.2649	+0.5	25.872	0.4986	0.4992	+0.6
16.620	0.3460	0.3467	+0.7	26.527	0.5112	0.5121	+0.9
16.997	0.3539	0.3539	0.0	27.749	0.5347	0.5354	+0.7
16.619	0.3460	0.3458	-0.2	26.401	0.5088	0.5078	-1.0
28.104	0.5851	0.5856	+0.5	15.230	0.2934	0.2942	+0.8
30.820	0.6417	0.6416	-0.1	16.415	0.3163	0.3173	+1.0

APPLICATION TO SEPARATION OF COPPER FROM TIN

The apparatus was tested on the separation of copper from tin. The electrode potentials of these metals are +0.345 and -0.136, respectively, and a separation of copper from appreciable amounts of tin by the ordinary electrodeposition process is impossible. The deposition was carried out from a hydrochloric acid solution containing hydroxylammonium chloride to prevent the liberation of chlorine at the anode, a procedure first described by Schoeh and Brown (4).

Standard solutions of copper and tin were prepared as follows:

Electrolytic copper prepared by electrolysis of c.p. copper sulfate was dissolved in dilute nitric acid and converted to copper sulfate by evaporation with a slight excess of sulfuric acid. The gray, anhydrous copper sulfate remaining after fuming was dissolved carefully and diluted. The copper in this solution was determined electrolytically. A weight buret was used to measure out the solution. To each determination were added 3 grams of ammonium nitrate and 3 ml. of nitric acid. The electrolysis was carried out in the usual way and continued sufficiently long to ensure the deposition of all copper. The results are given in Table I.

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St. Louis Plant of Fisher

Fisher Scientific Co. has established a plant at 2109 Locust St., St. Louis, Mo., to serve laboratories in the central states area with supplies. The 4-story building was engineered from its receiving platform to shipping room to expedite movement of orders. All floors and departments are furnished with modern equipment for efficient handling of merchandise and, as at Pittsburgh and New York, there is available a staff trained to render sales and technical service.



# Microdetermination of Sulfate

## A Colorimetric Estimation of the Benzidine Sulfate Precipitate

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IN CONNECTION with another investigation, the need arose for a simple, rapid, and accurate method for the determination of small amounts of sulfate, using only the limited equipment and reagents then available. The following method was developed and proved extremely satisfactory: The sulfate in the sample was precipitated as the benzidine sulfate, purified, and dissolved in 0.2 *N* hydrochloric acid, diazotized, and coupled, after destroying the excess nitrous acid, with Bratton and Marshall's reagent (1), *N*-(1-naphthyl)ethylenediamine dihydrochloride. The resultant intense purple color was read in a Klett-Summerson photoelectric colorimeter.

Colorimetric methods for the determination of sulfate are not new. Yoshimatsu (8) first treated the dissolved benzidine sulfate precipitate with a mixture of iodine, potassium iodide, and ammonia, and compared the resultant brown color with a benzidine sulfate standard similarly treated. Hubbard (2) and later Wakefield (7) used the yellow color produced by the action of hydrogen peroxide and ferric chloride on the dissolved benzidine sulfate precipitate. Kahn and Lieboff (3), thus far, were the only ones to couple diazotized benzidine sulfate with an alkaline solution of phenol. Lentonoff and Reinhold (4) used the color produced by the action of sodium  $\beta$ -naphthoquinone-4-sulfonate on an alkaline solution of benzidine sulfate as a basis for comparison.

Since its initial introduction by Bratton and Marshall (1) as a coupling agent for the determination of sulfonamides, *N*-(1-naphthyl)ethylenediamine dihydrochloride has found increasing popularity. Shinn (6) found that it gave superior results for the determination of nitrite in water, sewage, and foods. Recently, Looney and Dyer have used this reagent with success in the determination of potassium in serum (5).

### REAGENTS

Sodium nitrite, 0.1%, prepared fresh daily.

Ammonium sulfamate, 0.5%.

*N*-(1-naphthyl)ethylenediamine dihydrochloride, Eastman Kodak Company No. 4835, 0.1%. The solution is made up in distilled water and kept in a dark bottle. The reagent keeps best if refrigerated.

Benzidine hydrochloride reagent, 4.0 grams of pure benzidine hydrochloride dissolved in a small amount of distilled water and made up to 250 ml. with 0.2 *N* hydrochloric acid.

Standard Sulfate Solutions. Stock, 0.5437 gram of reagent potassium sulfate dissolved in distilled water and diluted to 1 liter; 1.0 ml. is equivalent to 0.3 mg. of sulfate. Dilute standard, 10.0 ml. of the stock standard diluted to 100 ml. with distilled water; 1.0 ml. is equivalent to 0.03 mg. of sulfate.

Standard Benzidine Hydrochloride. Stock, 0.4014 gram of pure benzidine hydrochloride dissolved in a small amount of 0.2 *N* hydrochloric acid and diluted to 100 ml. with the same solvent; 1.0 ml. is equivalent to 1.5 mg. of sulfate. Dilute standard, 1.0 ml. of stock solution diluted to 100 ml. with 0.2 *N* hydrochloric acid; 1.0 ml. is equivalent to 0.015 mg. of sulfate.

Acetone-ethanol, 1 to 1, using 95% ethanol and reagent acetone.

### PROCEDURE

To a 15-ml. centrifuge tube with a narrowed conical bottom, containing 1.0 ml. of glacial acetic acid and 1.0 ml. of benzidine hydrochloride reagent, an amount of sample containing between 0.015 and 0.15 mg. of sulfate is added, the solution is mixed, 2.0 ml. of acetone-alcohol are added, the tube is capped, and the contents are thoroughly mixed by rolling between the hands. The tube is allowed to stand in an ice-water bath for a half hour to complete precipitation.

At the end of this period, the tube is centrifuged at 2500 r.p.m. for 10 minutes and the supernatant poured off with one rapid motion. While still inverted, the mouth of the tube is carefully touched with a pad of clean filter paper to blot off excess liquid. The tube is permitted to drain on the filter paper pad for 5 minutes.

The walls of the tube are washed with 2.0 ml. of acetone-alcohol in a manner to avoid disturbing the tightly packed precipitate. The tube is recentrifuged for 5 minutes, and the supernatant poured off and permitted to drain for 5 minutes on the filter paper pad. The washing and draining procedure is repeated once more.

Two milliliters of 0.2 *N* hydrochloric acid are blown into the centrifuge tube. The precipitate dissolves almost instantly. The tube is placed in an ice-water bath, 1.0 ml. of sodium nitrite solution is added, and the tube is shaken to mix the solutions and allowed to stand for 3 minutes. One milliliter of ammonium sulfamate solution is added, and the tube is shaken and allowed to stand for an additional 2 minutes. Finally, 1.0 ml. of *N*-(1-naphthyl)ethylenediamine dihydrochloride solution is added to develop the color. After mixing, the violet solution is permitted to stand for 20 minutes, transferred quantitatively to a 50-ml. volumetric flask, and diluted to mark with distilled water. The solution is read in a photoelectric colorimeter using a green filter. The Klett No. 54 is satisfactory for the Klett-Summerson instrument.

It is recommended that a standard solution of benzidine hydrochloride or standard sulfate solution containing 0.03 mg. as sulfate be carried through, at the same time. The former solution need only be diazotized and coupled, while the latter must be carried through the entire procedure as described above. A blank, using 2.0 ml. of 0.2 *N* hydrochloric acid, diazotized and coupled with the color reagent, serves as zero reading on the photometer.

Table 1. Determination of Sulfate

SO <sub>4</sub> Mg.	SO <sub>4</sub> Recovered Mg.	Error Mg.	Error %
0.0150	0.0152	0.0002	1.3
0.030	0.0300	0	0
0.030	0.0303	0.0003	1.0
0.060	0.060	0	0
0.060	0.0597	0.0003	0.5
0.090	0.0921	0.0021	2.3
0.150	0.1470	0.0030	2.0

### CALCULATION

Reading of unknown  $\div$  reading of standard  $\times$  concentration of standard = mg. of sulfate in sample

### DISCUSSION

This method offers many advantages. The ease and simplicity with which the determination is carried out, together with the availability of the reagents used, are foremost. No extreme precautions are necessary beyond attention to the use of narrowed conical tips on the centrifuge tubes. These are readily fashioned by drawing the tips of ordinary glass centrifuge tubes in a flame until the diameter of the tip is about 2 mm. After centrifugation, the precipitated benzidine sulfate packs tightly and can be easily washed without disturbance or loss. Tubes which have been soaked in dichromate-sulfuric acid mixture must be carefully washed to remove all traces of sulfate.

The color obtained on final coupling with Bratton and Marshall's reagent has remarkable stability. In initial runs, stability studies revealed no decrease in intensity even after 12 hours. The color follows Beer's law closely. This is true of both solutions of pure benzidine hydrochloride and known sulfate solutions used in recovery studies. The precision of triplicate determinations was excellent, not varying by more than 0.1%.

The limitations of benzidine sulfate precipitation in the presence of large amounts of chloride and phosphate should be pointed out. At the time this study was carried out the sulfate concentration in a dialyzate which had no, or at most, traces of chloride



and phosphate present was being checked. This provided no interference. In the case of biological materials, phosphate should be completely removed. Chloride should not be present in amounts such that the weight ratio of chloride to sulfur exceeds 30; else, precipitation of benzidine sulfate is not complete.

The method is accurate in a range extending from 0.05 to 0.150 mg. of sulfate with a maximum error of 2%. The results obtained on pure sulfate solutions are summarized in Table I. Each value is the mean of triplicate determinations.

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## NOTES ON ANALYTICAL PROCEDURES

### An Observation of Possible Value for Sugar Determinations

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IN AN attempt to modify the Sichert and Bleyer reagent (1) for micro use, potassium iodide was included in the reagent. When this reagent was heated with glucose, a precipitate formed which was identified as cuprous iodide. The presence of the iodide ion speeds up the reduction reaction, presumably because of the removal of the cuprous ion from solution as cuprous iodide. The observation may have value for the determination of sugars, for the cuprous iodide can be determined iodometrically in the solution or separated and weighed directly. Its weight is of the order of ten times that of the glucose taken.

The reagent may be made up as follows: To 250 cc. of water are added 500 grams of hydrated sodium acetate, 75 cc. of 5% acetic acid, and 5 grams of potassium iodide. The solids are dissolved by warming the solution, and 25 grams of crystalline cupric sulfate are added as a 10% solution. About 40 grams of glucose are now added, the volume is made up to 1 liter, and the mixture is heated in boiling water for 45 minutes, let cool slowly, and filtered to remove the crystalline precipitate of cuprous iodide which had separated during the heating. For the determination, the solution is heated for 30 minutes at 100° with an equal volume of glucose solution.

Further work on this reagent is not contemplated.

Lowering of the pH by the addition of more acetic acid results in the formation of less precipitate for a given amount of glucose; if the amount of acetic acid is decreased, a white precipitate (apparently cupric hydroxide or a basic cupric salt, as it is nonreducing) is formed on heating, even in the absence of glucose. Omission of the preliminary heating with glucose causes a slight black precipitate to form during the determination if the quantity of glucose being determined is very small. The use of more than 5 grams of potassium iodide per liter increases the speed of the reaction, but makes iodometric estimation of cuprous iodide in the solution difficult. Less than 0.2 gram of glucose will give no reduction. The reagent is affected by maltose, whose reduction, under the conditions mentioned, is about  $\frac{1}{6}$  that of an equal weight of glucose. The maltose reduction is not complete even after 50 minutes of heating, whereas the glucose reduction shows no increase after 30 minutes.

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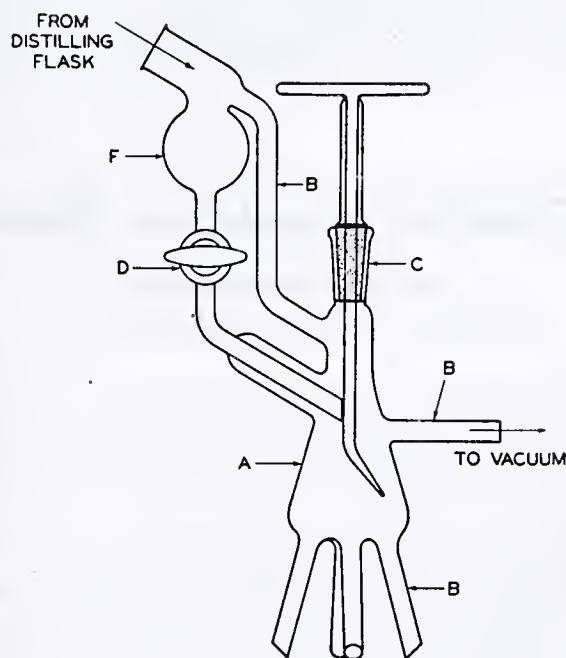
This work was aided by a grant from Mead Johnson and Co.

### Improved Distillation Receiver

HOWARD M. WADDLE<sup>1</sup>

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THE usual variety of cow's-udder fraction cutter (1) has the inherent disadvantage that each drop of distillate must be collected in one of the receivers attached to the cutter. The modification described makes it possible to interrupt the flow of a distillate while the operator follows the course of a temperature change in the fractionating column. Since the fraction cutter can be clamped in a stationary position, it is possible, in the case of low-boiling fractions, to surround the entire receiver with a freezing mixture.



In practice bulb *F* is approximately 10 ml. in diameter. Ground joint *C* has a standard taper 14/35, and the body, *A*, is made from a 50-ml. Erlenmeyer flask. All glass tubing, *B*, is 8 mm. The inside rod that conducts the distillate to the receivers must rotate freely and must not leave the surface of the tube coming from stopcock *D*, 2-mm. bore.

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# A Modification of the Ethanolamine Hydrolysis Method for Determination of Methyl Bromide

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THE study of the insecticidal value of methyl bromide as a fumigant requires a method for determination of this compound mixed with air. A modification of the method described by Stenger *et al.* (1) has been developed for its determination in the atmosphere of chambers during fumigations.

The sampling methods described by Stenger *et al.* (1) for various reasons could not be applied directly under some of the conditions which the authors encountered in their work, which required taking successive samples from several locations simultaneously. The modification developed involves withdrawal by aspiration of a portion of the atmosphere of the chamber, passing it through a series of absorption tubes packed with sand wet with ethanolamine, and subsequent determination of the bromide ion by the Volhard method. In this way gas losses during sampling are avoided.

Various types of absorption tubes may be used. The one which the authors found most satisfactory was made from 1-cm. (inside diameter) glass tubing bent in the form of a V, with the bend flattened so that the packing could be retained in one arm. The sand used for packing was of such a size that it would pass through a 10-mesh screen but be retained on a 16-mesh screen. It was prepared for use by digesting with concentrated nitric acid, washing free of acid, and igniting. The absorption tubes, each containing a column of sand 25 cm. high, moistened with 2 ml. of ethanolamine, were connected by means of inverted U-tubes, and all joints were rubber-covered glass to glass. The number of absorption tubes in series is dependent upon the concentration of methyl bromide and the rate of aspiration. The authors used four absorption tubes and a sampling period of 20 minutes. A 2-liter sample was taken for a concentration of 453.6 grams per 28,320 liters (1 pound per 1000 cubic feet) and a 1-liter sample for concentrations of 0.9 and 1.8 kg. (2 and 4 pounds). Usually more than 70% of the methyl bromide was

recovered from the first absorber, about 20% from the second, 6% from the third, and 2% from the fourth.

Following sampling, the contents of the absorption tubes were washed into Erlenmeyer flasks, and the bromide ion was determined by the Volhard method. Since ethanolamine retards the end point, a blank on the same amount of ethanolamine should be carried through the procedure and allowed for in calculating the results.

This method has given results reproducible with a standard deviation of  $\pm 0.01$  pound with methyl bromide concentrations of approximately 1 pound per 1000 cubic feet.

The amounts of methyl bromide recovered from a fumigation chamber of 1000 cubic feet capacity ranged from the equivalent of 96 to 100% of a 1-pound charge (mean recovery 97%), 95 to 100% of a 2-pound charge (mean recovery 97%), and 91 to 98% of a 4-pound charge (mean recovery 95%). The samples were taken 15 minutes after introduction of the fumigant. However, this chamber was exposed to the effect of wind. A statistical analysis was made, using the results obtained from 88 individual samples, to calculate the normal rate of leakage and the increased rate due to wind of different velocities. Using the calculated concentrations in the chamber at the time of sampling and comparing with the analytical results, the recoveries were between 1 and 2% greater than those presented.

## ACKNOWLEDGMENT

The authors are indebted to W. E. Fleming for the statistical analysis of certain of the data.

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# A Simplified Fritted-Glass Bubbler

RICHARD KIESELBACH

Bakelite Corporation, Bound Brook, N. J.

IN THE course of work involving the absorption of gases, a need was felt for a fritted-glass bubbler having the following advantages not to be found in the conventional jar type of bubbler.

There should be no dead spaces in the bubbler, so that a minimum of absorbing solution could be used effectively. A minimum of wash water should be required to wash out the solution at the end of a run. The possibility of loss of liquid while draining and washing should be reduced to a minimum. It should be possible to connect a series of bubblers quickly and easily, without the use of rubber tubing, and without difficulties of alignment. The bubbler should preferably be compact, rugged, and inexpensive.

The bubbler shown in the illustration proved to be the answer to the problem, and is of the simplest possible design. When a gas flow rate of 300 ml. per minute is used, the bubbler operates efficiently with as little as 15 ml. of absorbing solution. For special applications, the dimensions could, of course, be altered.

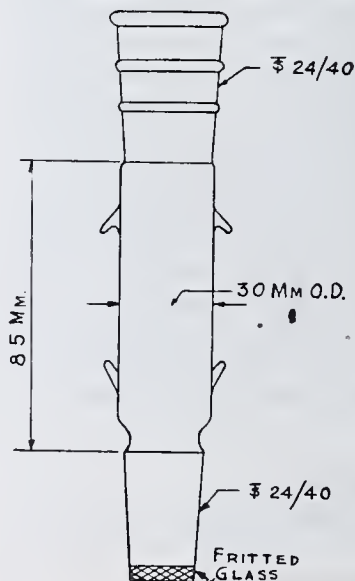
In operation, a series of bubblers is set up in the following manner: The No. 1 bubbler is inserted in the standard taper neck of a flask bearing a tubulature for the entrance of the gas. A slight

pressure (lung power is enough, where permissible) is applied at the tubulature, and the absorbing solution poured into the bubbler. The tubulature is closed by means of a stopcock or pinchclamp to maintain the pressure, and a second bubbler inserted in the neck of the first. The process is repeated for as many bubblers as are required, and the apparatus is ready for use.

At the completion of a run, a suction is applied at the tubulature of the flask, pulling the solution into the flask. A very

small amount of water from a wash bottle is required to wash down the bubblers, this being sucked down in the same way. Obviously, loss of liquid is next to impossible at this stage. Ideally, the receiving flask should be so designed that it can be used as a vessel for subsequent reactions.

This type of bubbler has one disadvantage—the necessity of maintaining a gas pressure while filling, and until putting into operation—which may preclude its use in certain applications. For many purposes, however, this inconvenience is more than compensated for by the advantages listed above.





## Apparatus for Washing Selas Crucibles

C. R. SCHLEY

Lucky Heart Laboratories, Inc., Memphis, Tenn.

A RECENT article (1) outlines an arrangement for washing Selas filtering crucibles by reverse flow. This laboratory has performed this task in a slightly different manner, which the author believes to be more convenient and perhaps more economical of material.

The crucible to be cleaned is placed in an inverted position with the top fitting into a circular groove cut into the top of a Walter-type crucible holder held in a standard filter flask. The bottom of the crucible is covered with another holder of the same type from which the regular stem has been removed. A short-stemmed funnel is placed upright in the opening of the holder. The groove in the lower holder is filled with a suitable liquid, the crucible is pressed down as suction is applied to the filter flask, and the desired washing medium is poured into the funnel.

Strong acids or solvents which would damage the rubber connection should be added at such a speed that the volume does not build up in the reservoir, and the liquid runs through the filter as soon as it is added. Unnecessary damage to the rubber connections between filter flask and trap is also avoided by the stem in the lower holder which conveys the wash liquid down into the flask and prevents splashing into the side arm.

This arrangement utilizes equipment readily available and requiring no modification other than cutting the small groove in one of the holders, which in no way interferes with its subsequent use in the usual manner.

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## LABORATORIES and NEW EQUIPMENT

## BOOK REVIEWS

### Hotel Association Testing Laboratory

The American Hotel Association testing laboratory, which is part of the Department of Hotel Administration at Michigan State College, East Lansing, Mich., is located on the college campus. Bernard R. Proulx is head of the department and Bruce Hartsuch is in charge of the laboratory and all tests.

Established about a year ago, the laboratory is as yet meagerly equipped and uses considerable equipment from another department of the college, but when war conditions permit it is planned to equip it fully and make it one of the outstanding laboratories of the country. Tests of textiles and cleaning compounds, as well as other physical tests, are now being made.

### Specimen Holder for Abraser

A specimen table for the Taber abramer, that permits testing paper products for wear resistance in moist or wet condition, has been announced by Taber Instrument Corp., North Tonawanda, N. Y. Results of tests are reported either numerically as the number of wear cycles to produce a given amount of wear, or as loss in weight when weighed on a precision laboratory balance.

### Autoclave

A 1-gallon autoclave, made by the Industrial Machinery Co., Bloomfield, N. J., is now available for prompt shipment. It combines in its design, construction, and equipment the functions and usual features of large high-pressure autoclaves, sulfonators, nitratators, chlorinators, kettles, and similar equipment.

### Tin-Plated Panels

Bright tin-plated sheet steel panels, approximately 31 gage and measuring 3 × 5 inches, are available in limited amounts from the Stewart Research Laboratory, P. O. Box 173, Washington, D. C. They are used in flowout and flexibility tests of paints and varnishes, in kauri-reduction tests, and for filing samples for reference.

*Colorimetric Determination of Traces of Metals.* E. B. Sandell. xvi + 487 pages. 15 × 23 cm. Interscience Publishers, Inc., 215 Fourth Ave., New York, N. Y., 1944. Price, \$7.00.

This book is Volume III of "Chemical Analysis", the new series of monographs on analytical chemistry and its applications being published by Interscience Publishers. It is a worthy addition to their list, published or in preparation.

The author's aim is presentation of a "limited number of methods which . . . appear to be best suited for dealing with traces of metals. No one reagent is necessarily the best for the determination of an element in all kinds of samples or under all conditions, and consequently two or three methods are described. . . for a number of the metals. The treatment is to a considerable extent based on the experience of the writer . . ."

Part I is a general introduction. The four chapters deal with trace analysis (17 pp.), methods of separation (15 pp.), methods of measurement (40 pp.), and 19 general colorimetric reagents (36 pp.). Anyone not widely experienced in colorimetric methods of measurement will find much valuable information in this section. The chapter on measurement seems the weakest, but obviously the present range and variety of instruments could not be covered in the space available. A comprehensive critical discussion of this subject is definitely needed. In connection with the stated limit of range of colorimetric methods, Mehlig showed the practicability of working with far higher concentrations of iron and of copper in ores.

Part II, covering analytical procedures, includes methods for 45 separate metals and the rare earth elements. Since various metals (Ac, Cs, Hf, Ma, Pa, Ra, Rb, Sr, Th, Y) are omitted and only selected procedures are described, perhaps a more accurate title might have been "Selected Methods for the Colorimetric Determination of Certain Metals". It seems likely that many analysts will wish that here had been included methods for nonmetallic elements, such as the halogens, silicon, nitrogen, and phosphorus (those omitted are B, Br, C, Cl, F, H, I, N, O, P, Po, S, Se, Si, Te). A second volume might be devoted to these elements.

In general, the information for each element is classified under separations, methods of determination, and industrial applications, such as rocks, ores, minerals, metals, soils, water, and biological materials. Adequate operating directions are given for applying the



selected methods, and many references and notes document and amplify the material. As stated in the preface, balanced treatment is still impossible because of the lack of information on, and critical study of, many methods. Most colorimetric methods need such investigation.

In view of Professor Sandell's contribution to colorimetry in this meritorious book, the reviewer hesitates to offer an adverse comment. The following statements and questions are included, therefore, with the sole aim of helping to clarify and improve analytical literature. Grid lines are generally desirable in using graphs, and observed points should be shown if possible. One likes to know the spectral band width used in determining curves such as that for the permanganate solution (see Figure 4, which does not show the characteristic small bands. Why?). Consistency is desirable in formulating the heteropoly compounds, preferably in accordance with Keggin's work (including naming in terms of the central atom). There is possible uncertainty regarding the plotting of the spectral curves because of the use as ordinate legends of extinction,  $\log I_0/I$ ,  $\log \epsilon$ , transmission, transmittancy, and absorption. Is it transmission or transmittancy (the terms are not synonymous)? Is it  $\epsilon$  or  $\log \epsilon$  (Figure 27)? Is it absorption—that is,  $100 - T$  (Figure 49)? Is transmittancy a logarithmic value (Figure 64)? Is it not simpler to plot transmittancy directly on semilogarithmic paper, rather than  $\log 1/T$  (or  $\log I_0/I$ ) on linear paper, for curves to test conformity to Beer's law or to use in determinations?

The author's justified caution about using others' extinction coefficients (p. 57) implies the necessity of determining one's own calibration data for any specific instrument.

Very few typographical errors were noted. Optical density is meant on page 34. The symbol  $T_i$  appears twice in the table on page 75. Although hardly an error, the use of the abbreviation "etc." leaves something to be desired from the standpoint of concise scientific writing.

Conversations with industrial analysts have confirmed the reviewer's initial opinion that this book is now the first compilation to which to turn in the hope of finding a tested colorimetric method, clearly presented for use. Analytical chemists are indeed indebted to Professor Sandell for such a monograph.

M. G. MELLON

**Experimental Spectroscopy.** *Ralph A. Sawyer.* 323 pages. Prentice-Hall, Inc., New York, N. Y. Price, \$5.00.

This book fills a long-felt need for a general text and reference book on applied spectroscopy. The author deals with spectroscopy as a tool for the chemist or physicist, and not as a science in itself. The primary emphasis is placed on those principles and techniques that are fundamental to the applications of spectroscopic equipment as a general research tool. Highly theoretical topics are avoided and no attempt is made to cover phases of experimental spectroscopy requiring highly specialized knowledge of mathematics or atomic physics. The origin of spectra, the Raman effect, absorption spectrophotometry, and similar topics of rather limited interest or requiring extensive mathematical treatment are largely omitted.

A brief historical sketch of the development of spectroscopy and a short nontechnical discussion of light sources make up the first two chapters. Chapters 3 to 7 provide the most complete, readable presentation of the principles of design, adjustment, and use of prism and grating spectrographs which has appeared in a single book or paper. If the book contained nothing else of merit, this section alone would make it a valuable contribution to spectroscopic literature. The reviewer could not escape the impression that the author underestimates the proportion of spectrographs employing original gratings now in use. Dr. Sawyer's industrial experience being largely limited to prism spectrographs, he is apparently not fully aware of the fact that spectrographs employing original gratings are now produced in greater quantity than prism spectrographs in this country.

Chapter 8 covers the photographic process as used in spectrography. The author makes no attempt to deal with the theoretical aspects of photographic chemistry and presents little new material on the application of photography to spectroscopy. However, the familiar properties and behavior of the emulsion are adequately covered and correlated with spectroscopic applications. Interesting and useful data, assembled from previous publications of Kodak Research Laboratories, are presented.

The determination of wave length is treated in some detail in Chapter 9, with particular attention to practical methods based on comparison with primary, secondary, and tertiary standards. Conventional methods of extrapolation are discussed and the practical methods of identifying lines in analytical work are described. References to all the most important sources of wave-length data are given. Fundamental methods of wave-length determination, such as interferometry, are mentioned only casually.

Chapter 10, dealing with the measurement of light intensity, provides an excellent general treatment of the problem of photometry in both emission and absorption spectroscopy and covers both visual and objective instrumental methods of photometry. Valuable notes on technique and sources of error are included. A number of commercially available microphotometers are described and, while these descriptions are for the most part reasonably accurate, the author was apparently unfamiliar with one of the makes discussed, the Applied Research Laboratories comparator-densitometer. The author classified this instrument as a projection-type instrument and describes a projection-type microphotometer as one in which "a considerable length of the spectrum is projected on a screen carrying an adjustable slit, behind which the light-sensitive element is placed". The Applied Research Laboratories instrument is not a projection instrument in this sense and employs projection only as a means of providing the comparator feature and locating the line to be measured. Moreover, the film or plate is not moved during the scanning of the line, as implied by the author, and the remarks concerning the relative advantages and disadvantages of the projection-type instrument do not strictly apply to this microphotometer. Aside from this rather minor confusion, the treatment of photometers is unbiased, accurate, and effective.

The last three chapters, 11, 12, and 13, deal with infrared spectroscopy, vacuum ultraviolet spectroscopy, and "spectrochemical" analysis, respectively. These chapters give a brief but reasonably complete treatment of the practical aspects of the selection and design of the instruments and the fundamental techniques employed.

The reviewer noted several deviations from currently accepted spectroscopic nomenclature. Among the words or phrases used, to which the scientific lexicographer might take exception, are "linear dispersion" and "spectrochemical analysis". The author's use of such words and phrases detracts infinitesimally from the value of the book and is objectionable only because the book will undoubtedly become one of the standard references on the subject and is likely to be widely quoted.

Not only is the book valuable for the specific spectroscopic information it contains, but also it provides an excellent bibliography. The references given are well selected and few important references are omitted. The bias and prejudices common to most discussions of spectroscopic apparatus and techniques are notably absent in this work, and the author's impartial factual treatment of the controversial phases is highly praiseworthy. Remarkably complete, considering the scope of the subjects embraced, the book should be of great help in broadening the knowledge and interests of workers already in the field. While highly theoretical phases have been avoided, the author presents sufficient fundamental theory and correlates it so well with practical considerations that the book should contribute towards rationalizing the now overly empirical art of spectroscopy.

J. RAYNOR CHURCHILL

**Experiments in General Inorganic Chemistry.** *J. L. Maynard and T. I. Taylor.* 550 pages. D. Van Nostrand Co., 250 Fourth Ave., New York, N. Y., 1944. Price, \$2.90.

A laboratory manual designed to accompany "General Inorganic Chemistry" by Sneed and Maynard, which may be adapted for use with any standard textbook by changing the order of experiments. Laboratory techniques and descriptive aspects of chemistry are emphasized.

## Specifications for Analytical Reagents

A limited number of planographed copies of the first supplement to "A.C.S. Analytical Reagents" [IND. ENG. CHEM., ANAL. ED., 16, 281 (1944)] are available on application from INDUSTRIAL AND ENGINEERING CHEMISTRY, 1155 Sixteenth St., N. W., Washington 6, D. C.



# Systematic Procedure for Identification of Synthetic Resins and Plastics

T. P. GLADSTONE SHAW

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The procedure given will identify most resins of commercial importance at the present time. Once the resin has been isolated in as pure a condition as possible it is necessary to determine to which of eight groups it belongs, then to proceed systematically to its tentative identification. Confirmatory tests are applied to confirm or disprove this. In the latter event, or if short cuts are possible because of the history of the resin, use is made of a classification of the properties for the various general types of resins.

EVERY chemist employing resinous substances is confronted at some time with the task of identifying a resin. No detailed procedure has so far appeared which would enable a systematic attack on this problem to be made, although a number of methods suitable for a few common resins are known (1, 10, 13). The method given in this paper has proved very serviceable in these laboratories for the past two years.

This method of analysis depends upon using a single resin for the group and systematic procedures. The general order of procedure to be followed on an unknown sample is:

1. Separation of the resin or resins from the solvents, plasticizers, fillers, pigments, and dyes
2. Separation of mixtures into individual resins
3. Classification of the separated resin according to the group tests
4. Identification by following the scheme for the group into which the resin falls, so as to arrive at the probable identity of the resin
5. Confirmation by specific tests

Cases will occur where the confirmatory tests do not yield a clear-cut identification. This may be due to imperfect separation of a mixture or the presence of a resin which is not covered in this paper. Some general reactions are given below which will help to identify the type of resin in such cases.

The scheme will function with the particular resins used and with allied resins; but it will not distinguish between different degrees of polymerization of the same monomer. It has not been possible to cover all the resins for each type; therefore, it is necessary to confirm the identifica-

tion. Where the same resin is listed in different places in the tables it is because of similarity in properties of related resins which could not be distinguished without use of the trade name, or a few borderline cases which might fall in both places.

### PREPARATION OF SAMPLE

In most fields there are published methods (2, 8, 14, 16) covering the separation of vehicles, pigments, or plasticizers from the resinous constituents that may be encountered. It is beyond the scope of this paper to do more than briefly indicate the methods employed.

REMOVAL OF VEHICLES AND PLASTICIZER. In handling a solution, addition of a nonsolvent such as ligroin or water to precipitate the resins is useful. The plasticizer will usually be left

Table I. Separation of Groups

(Sample previously separated from solvents, plasticizers, fillers, pigments, dyes, and other resins)

Halogens
Strongly positive: Test according to Group A.
Negative: Test for nitrogen and sulfur.
Nitrogen and sulfur
Nitrogen positive, sulfur negative or very weakly positive: Test according to Group B.
Nitrogen and sulfur positive: Test according to Group C.
Nitrogen negative, sulfur positive: Test according to Group D.
Nitrogen and sulfur negative: Test for saponification number.
Saponification No.
Over 325: Test according to Group E.
120 to 325: Test according to Group F.
Less than 120: Test for acetyl number.
Acetyl No.
Over 40: Test according to Group G.
Less than 40: Test according to Group H.

Table II. Separation of Group A

(Halogens present)

#### A. Test solubility in ligroin

Soluble: Chlorinated diphenyls. Confirm by Test VI. Melt with very little decomposition. High refractive index

#### Insoluble: Test solubility in hot acetone

Soluble: Polyvinyl chloride-acetate copolymers. Medium acetate type. Confirm by Test II

#### Insoluble: Test solubility in ethyl acetate

Soluble: Chlorinated rubber. Confirm by Test III

#### Insoluble: Test solubility in ethylene dichloride

Soluble: Polyvinyl chloride or low acetate polyvinyl chloride-acetate copolymers. Confirm by Test II

Insoluble: Test solubility in pyridine (B)

#### B. Solubility in pyridine

Soluble: Test solubility in tetrachloroethane

Soluble: Polyvinyl chloride-acrylate copolymer. Hard resin. Low refractive index

Insoluble: Cashew nut oil polymer. Confirm by Tests II and VI. Soft sticky black resin

#### Insoluble: Test solubility in tetrachloroethane

Soluble: Rubber hydrochloride. Confirm by Test VI

#### Insoluble: Test solubility in morpholine

Solvent and resin turn black. Polyvinylidene chloride resins. Confirm by Test II

Insoluble: Chloroprene rubbers. E type soluble in dioxane, other type insoluble. Confirm by Test II. Sulfur usually present



Table III. Properties of Resins in Group A

Polyvinyl Resins											
Test No.	Property Sought	Chloride	Chloride-Acetate		Chloride-acrylate	Polyvinyl- idene chloride	Chlorinated Diphenyls	Chloroprene	Rubber Substitutes		Cashew nut oil polymer
			Low acetate type	Medium acetate type					Chlorinated rubber	Rubber hydrochloride	
I	Solubility										
	Acetone	I	IG	S	I	I	S	I	IG	I	I, Sl, G
	Ethyl acetate	I	I	S	I	I	S	I-S	S	I	I, Sl, G
	Dioxane	S	S	S	I	I	S	I	S	I	IG
	Ligroin	I	I	I	I	I	S	I	S	I	I
	Benzene	I	S	S	IG	I	S	I-IG	S	I	IG
	Ethylene dichloride	S	S	S	S	I	S	I-IG	S	I	S
	Pyridine	IG	....	I	I	I	S	I	I	I	I
	Acetic acid	I	....	....	IG	I liquid, resin turn black	....	I-IG	....	I	IG
	Morpholine	Sl, S.; G	....	....	S	I	S	I-IG	S	S	IG
II	Tetrachloroethane	IG	....	S	S	Resin slowly turns yellow	Neg.	Red-brown	Neg.	Neg.	Red brown to dark brown
	Liebermann-Storch	Resin slowly turns blue	Resin slowly turns green to blue to brown	Resin slowly turns green to blue to brown	Neg.						
III	Carbonate fusion										
	Odor	(Resembles that of smoke from wet wood)			Gunpowder	Aromatic	None	Wet wood	Hypochlorite	Sl. rubbery	Oily
	Char	Pos.	Pos.	Pos.	Pos.	Pos.	None	Pos.	Pos.	Pos.	Pos.
	Fume	V. Sl.	V. Sl.	V. Sl.	Heavy	V. Sl.	None	Sl.	V. Sl.	Sl. white	Heavy
V	Carboxylic esters	Neg.	Neg.	Neg.	Neg.	....	Neg.	....	Neg.	....	....
VI	Odor on ignition	Pungent	Pungent	Pungent	Pungent	Pungent	Sl. acid	Pungent and tarry	Pungent	Pungent	Oily
	Char	Pos.	Pos.	Pos.	Pos.	Pos.	Neg.	Pos.	Sl.	Pos.	Pos.
	Fume	Heavy yel.	Cons. yel.	Cons. yel.	V. heavy yel.	Nil	Neg.	Heavy brown	White	Heavy yel.	Heavy yel.
	Distillate	Nil	Nil	Nil	Nil	Nil	Much	Sl.	Nil	Sl.	Sl.
XIII	Acetates	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	....	Neg.	....	Neg.
	Refractive Index 20° C.	1.56	1.53	1.53	....	1.61	1.61-1.71	....	1.56	....	....
	20° C.	1.2-1.6	1.35	1.35	1.38	1.6-1.75	1.34-1.95	1.14-1.24	1.64	....	....
	Specific Gravity 20° C.										
Key to Abbreviations											
Cons., considerable or considerably											
G, gel											
I, insoluble											
Neg., negative											
Pos., positive											

in solution. If test-tube experiments show this method is not feasible, the solution may be poured into boiling water or dried by evaporation. Both the latter methods leave the pigments and plasticizers in the resins and they will require further treatment.

Oily vehicles may usually be removed by precipitation of the resin by a nonsolvent.

PIGMENTS AND FILLERS. Finely divided pigments sometimes offer considerable difficulty. If the pigment is held by Alundum or paper thimbles a Soxhlet extraction of the vehicle-free resins by a solvent will do. At times repeated filtration in the presence of filter aid such as Filter-Cel on a suction or pressure filter, using a very dilute solution in a low-viscosity solvent, is required. Centrifuging will serve in some cases.

DYES. Dyes are even worse to handle by precipitation by a nonsolvent, a procedure which must be repeated several times for removal of vehicles and plasticizers, usually leaves most of the dye in solution. Preliminary tests often indicate a solvent for the dye which has sufficient swelling action on the resin for a Soxhlet extraction of the dye to be made.

### MIXED RESINS

Where a mixture of resins is suspected these must be separated into as pure fractions as possible by suitable extraction or precipitation procedures designed to meet the particular case in hand. Fractions so separated, dried, and free from solvent are treated independently by the methods given below.

### METHODS FOR SEPARATION OF GROUPS

The following tests are applied in the order given (Table I):

HALOGENS. Beilstein's copper wire test is convenient (9, 11, 12). All substances in Group A give a strong test. A faint test may be ignored as due to volatile impurities or salts. It is necessary to make sure that some of the resin actually enters the flame with the wire.

Halogens may also be detected in a portion of the filtrate from the sodium fusion by acidifying and boiling with nitric acid, then adding silver nitrate.

NITROGEN. The usual sodium fusion (9, 11, 12) with the development of Prussian blue in the presence of ferric salts is used.

SULFUR. A drop of the filtrate from the sodium fusion applied to a silver coin quickly develops a dark stain in the presence of sulfur.

ACID NUMBER. While not required for group separation, this figure is conveniently obtained at this time and is useful as a confirmatory figure later.

Accurately weigh about 1 gram of resin and place it in 100 cc. of neutral dioxane, alcohol or other suitable solvent. Then warm gently under an air condenser for about 1 hour. Titrate the free acid with aqueous 0.1 N sodium hydroxide, using phenolphthalein indicator.

Acid number = 
$$\frac{56.1 \times \text{normality of NaOH} \times \text{cc. of NaOH used}}{\text{weight of sample}}$$

SAPONIFICATION NUMBER. To the neutral solution above, in a soft-glass flask, add 25 cc.



Table IV. Separation of Group B

(Halogens absent, nitrogen present, sulfur absent)

Apply Test IV	No blue color: Apply Test XI		Positive: Violet color. Apply Test XII	
Blue color: Nitrocellulose or nitrocellulose acetate. Distinguish by rate of burning; acetate may be identified by method of Simmonds and Ellis (15).	Negative: Apply Test III	Odor like hot aniline, butadiene-acrylonitrile copolymer. Confirm by action of acetone	Negative: Test solubility in pyridine	Positive: Casein-formaldehyde resin. Confirm by weak sulfur test and Test VI
	Odor of NH <sub>3</sub> and burning hair. Polyamide resin. Confirm by Test VI. Note. Glue, gelatin, or albumenoids might appear here. Physical form, positive biuret reaction, solubility in hot water can be expected to indicate such substances.		Soluble: Melamine-formaldehyde resin. Confirm by Tests III and VI	Insoluble: Urea-formaldehyde resin. Confirm by Tests III and VI

Table V. Properties of Resins in Group B

Test No.	Property Sought	Nitrocellulose	Urea-Formaldehyde Resin	Melamine-Formaldehyde Resin	Polyamide Resin	Casein-Formaldehyde Resin	Butadiene-Acrylonitrile Copolymer	Gelatin Glue Coagulated Protein
I	Solubility							
	95% ethanol	P.S.G.	I	I	I	I	I	I
	Acetone	S	I	I	I	I	P.S.G.	I
	Ether	I	I	I	I	I	I	I
	Ethyl acetate	S	I	I	I	I	IG	I
	Dioxane	S	I	I	I	I	IG	I
	Pyridine	S	I	S	I	I	IG	I-P.S
	Acetic acid	S	I	S	I	I	I	I-S
	Carbon tetrachloride	I	I	I	I	I	I	I
	Tetrachloroethane	I	I	P.S	I	I	IG	I
	Benzene	I	I	I	I	I	IG	I
	Hot water	..	..	..	..	..	..	S-I
II	Liebermann-Storch	NO <sub>2</sub> evolved	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
III	Carbonate fusion							
	Odor	Burning paper	NH <sub>3</sub>	Formaldehyde	NH <sub>3</sub> + burning hair	NH <sub>3</sub> + burning hair	Like hot aniline	Burning hair and NH <sub>3</sub>
	Char	Pos.	Pos.	Pos.	Pos.	Pos.	Nil	Pos.
	Fume	Sl.	Nil	Nil	Sl.	Sl.	Cons. white	V.Sl.
IV	Nitrates	Blue	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
VI	Odor on ignition	.....	NH <sub>3</sub> + burning hair	Formaldehyde	NH <sub>3</sub>	Burning hair	Like hot aniline	Burning hair
	Char	.....	Pos.	Pos.	Pos.	Pos.	Pos.	Pos.
	Fume	.....	V.Sl.	Sl. white	Sl.	Heavy yel.	Heavy yel.	Heavy
	Distillate	.....	Nil	Nil	Sl.	Sl.	Cons.	Nil
XI	Formaldehyde	Red	Purple	Strong red violet	Neg.	Pale violet	Neg.	Neg.
XII	Xanthoproteic	....	Neg.	Neg.	Neg.	Pos.	.....	Weakly pos.
	Refractive index 20° C.	1.50	1.55	1.55	1.54	.....	1.52	.....
	Specific gravity 20° C.	1.4	1.16	1.16	1.1	1.35	0.98-1.01	.....

Table VI. Separation of Group C

(Halogens absent, nitrogen and sulfur present)

Aqueous 0.5 N sodium hydroxide and warm under reflux until the resin is all in solution but not less than 1 hour. If the resin does not dissolve, allow to reflux overnight. Titrate excess sodium hydroxide with 0.5 N hydrochloric acid, using phenolphthalein or other indicator. For very dark solutions "Universal indicator" has proved useful. Where the resin is undissolved, vigorous shaking during titration is required to neutralize alkali absorbed by the swollen resin.		Test solubility in hot water		
		Soluble: Gelatin. Confirm by Tests III and XI	Insoluble: Apply Test III	
			Odor of burning hair. Casein-formaldehyde resin. Confirm by Tests VI, XI, and XII	Odor like hot aniline. See butadiene-acrylonitrile copolymer in Group B
			Odor of formaldehyde. Sulfonamide resins. Resins vary from viscous fluid to low-melting solids. Confirm by Acid No., Saponification No., and Test XI	

$$\text{Saponification number} = \frac{56.1 \times \text{normality of NaOH} \times \text{cc. of NaOH used by sample}}{\text{weight of sample}}$$

**ACETYL NUMBER.** Weigh about 2 grams of the sample accurately and place it in a Pyrex Erlenmeyer flask, add 20 cc. of pyridine-anhydride reagent, and place on a steam bath under an air condenser. Treat a blank on the reagents similarly. When all is in solution or after several hours if the resin only swells, add 5 cc. of neutral ethylene dichloride or benzene, stopper the flask, and shake vigorously. The resin should be dissolved or well broken up by this treatment. Add 100 to 150 cc. of distilled water and titrate with 0.5 N sodium hydroxide, using about twice the usual amount of phenolphthalein indicator. The titration requires vigorous shaking to remove the acid from the solvent layer and the red color should be permanent for at least one minute.

**REAGENT.** 880 cc. of pyridine (Barrett's 2A grade), 120 cc. of 95% or better acetic anhydride.

$$\text{Acetyl number} = \frac{(\text{cc. of NaOH blank} - \text{cc. of NaOH sample}) \times 56.1 \times \text{normality of NaOH}}{\text{weight of sample}}$$

#### TESTS USED IN SCHEMATIC PROCEDURE AND FOR CONFIRMATION

**TEST I. SOLUBILITY.** Place about 1 gram of sample in a test tube with 10 cc. of solvent, shake at room temperature several

hours, then note carefully whether the resin is soluble, partly soluble, or insoluble, or whether there is any color in the solvent or resin.

**TEST II. LIEBERMANN-STORCH REACTION.** Place a small fragment of resin on a spot plate and cover with a few drops of acetic anhydride. Now add 1 drop of concentrated sulfuric acid, so that it enters the liquid. Note the color reactions in the liquid and on the resin surface. Observe over a period of half an hour. List the colors in the order of their formation.

**TEST III. ODOR ON CARBONATE FUSION.** This test suppresses the acid constituents in the volatile decomposition products and allows some odors to be more readily recognized.

Fuse a piece of resin with 1.25 cm. (0.5 inch) of anhydrous sodium or potassium carbonate in a test tube. Note odors, fumes, and tendency to char.

**TEST IV. NITRATE.** Dissolve a few crystals of diphenylamine in about 0.5 cc. of 90% sulfuric acid and place a drop of this reagent on a piece of the resin on a spot plate. An immediate intense blue color indicates nitrocellulose or esters such as cellulose nitroacetate. Even a few per cent of nitrocellulose in another resin will yield this test, but the color will develop more slowly.



Table VII. Properties of Resins in Group C

Test No.	Property Sought	Sulfonamide Resins <sup>a</sup>			Gelatin, Glue, Albumenoids	Casein-Formaldehyde Resin
		M.S.	M.H.P.	K		
I	Solubility					
	95% ethanol	S	S	S	I	See under Group B. Sulfur test weakly positive
	Acetone	S	S	S	I	
	Ether	P.S	S	SL.S.G.	I	
	Ethyl acetate		S	P.S.G.	I	
	Dioxane	S	S	S	I	
	Pyridine	S	S	S	I	
	Acetic acid	S	S	S	I	
	Carbon tetrachloride	I	I	S	IG	
	Tetrachloroethane	S	S	IG	I	
	Benzene	S	S	S	I	
	10% NaOH	P.S	S	S	P.S	
	Hot water	I	I	I	S	
II	Liebermann-Storch	Neg.	Neg.	Neg.	Neg.	
III	Carbonate fusion					
	Odor	Formaldehyde	Formaldehyde + aromatic	Formaldehyde + aromatic	Burning hair + NH <sub>3</sub>	
	Char	Pos.	Pos.	Pos.	Pos.	
	Fume	Sl.	Sl.	Sl.	V.Sl.	
VI	Odor on ignition	Formaldehyde	Formaldehyde + aromatic	Formaldehyde	Burning hair	
	Char	Pos.	Pos.	Pos.	Pos.	
	Fume	Sl.	Sl.	V.Sl.	Heavy	
	Distillate	Sl.	Sl.	Sl.	Nil	
XI	Formaldehyde	Deep violet	Deep violet	Violet	Neg.	
	Refractive index 20° C.	1.59	1.596	1.56	.....	
	20° C.					
	Specific gravity 20° C.	1.36	1.35	1.31	.....	

<sup>a</sup> Monsanto designations. M.S., soft, low melting point solid. M.H.P., solid, softening about 62° C. K, viscous fluid.

Table VIII. Separation of Group D<sup>a</sup>

(Halogens and nitrogen absent, sulfur present)

Test solubility in pyridine

Soluble:	Organic polysulfides. Confirm by Test VI	Insoluble: Test solubility in carbon tetrachloride
		Soluble: Isobutylene copolymer with diolefins. Confirm by weak sulfur test, Test VI, and solubility in tetrachloroethane and benzene
		Insoluble: Vulcanized rubber. Confirm by Test VI. Beware of compounded butadiene-styrene copolymer. Odor of Test VI not strong with this resin

<sup>a</sup> Some crude dark cumarone resins and possibly others will show strong test for sulfur. If solubilities show substance definitely foreign to this group, ignore sulfur test and proceed to test for other groups.

TEST V. CARBOXYLIC ESTERS (5). Place a small piece of resin in a clean test tube and add 1 cc. of 6% alcoholic (water white) sodium or potassium hydroxide, then 1 drop of a saturated alcoholic solution of hydroxylamine hydrochloride. Shake and let stand 5 minutes. Heat for about 30 seconds while boiling, add 1 drop of 1% aqueous ferric chloride solution, and add carefully just sufficient 10% aqueous hydrochloric acid to dissolve the ferric hydroxide precipitate; then cautiously add a few drops in excess. A strong violet color indicates carboxylic acid esters. The color may be so strong that dilution with water is necessary in order to note it.

Too much hydrochloric acid will destroy the color and is to be avoided, but in negative tests a sufficient excess should be added (1 cc.) drop by drop to remove all doubt of the possibility that it is insufficient.

TEST VI. ODOR ON IGNITION. Heat a piece of resin strongly in a test tube, and note odor, fumes, charring, and presence of distillate.

TEST VII. TEST FOR PHTHALATES. Heat about 1 gram of resin with about 2 to 3 grams of pure phenol plus 5 drops of concentrated sulfuric acid until the melt turns orange or brown. Cool, dilute with water, and render alkaline with 10% aqueous sodium hydroxide. Characteristic red color of phenolphthalein indicates the presence of phthalates.

This test is preferred to the fluorescence test for phthalates and is less subject to error due to inexperience.

TEST VIII. TEST FOR PHENOLIC RESINS. This is the reverse of the test for phthalates. Heat about 1 gram of resin with about 1 gram of phthalic anhydride and 3 drops

Table IX. Properties of Resins in Group D

Test No.	Property Sought	Organic Polysulfide Rubber	Vulcanized Rubber	Isobutylene Copolymer with Diolefins
I	Solubility			
	95% ethanol	I	I	I
	Acetone	I	I	I
	Ether	I	I	I
	Ethyl acetate	I	I	I
	Dioxane	S	I	I
	Pyridine	S	I	I
	Acetic acid	I	I	I
	Carbon tetrachloride	I	I	S
	Tetrachloroethane	S	I	S
	Benzene	I	I	S
II	Liebermann-Storch	Pale red violet to brown to dark red brown	Neg.	Neg.
VI	Odor on ignition	Mercaptan + pungent	Burning rubber	Sl. aromatic
	Char	Pos.	Pos.	Sl.
	Fume	Heavy yel.	Heavy yel.	Cons. white
	Distillate	Cons.	Nil	Sl.
	Refractive index 20° C.	.....	1.52	1.52
	20° C.			
	Specific gravity 20° C.	1.34	1.1-1.18	0.92
	20° C.			

Table X. Separation of Group E

(Halogens, nitrogen, and sulfur absent. Saponification Nos. over 325)

Apply Test II

Resin slowly turns green

Saponification Nos.	325-500, polyvinyl alcohol - acetate, with high polyvinyl acetate. Acetyl No. high	Saponification Nos. 500-700, polyvinyl esters. Apply Test XI
	Red color, polyvinyl acetate. Confirm by characteristic odor. Test VI	Negative or pale violet or orange, vinyl ester copolymers

Resin slowly turns slightly brown.

Apply Test V	Red violet color, cellulose acetate. Confirm by Tests VI and XI. Insoluble in benzene	Negative, polymethyl acrylate. Confirm by Test VI. Soluble in benzene
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Color of liquid changes to deep orange, polybasic acid from rosin. Confirm by Tests VI and XI. Solution in hot phenol and 3 drops of H<sub>2</sub>SO<sub>4</sub> gives brilliant red which disappears on adding NaOH

Negative. Apply Test XI

Strong red, cellulose acetate. Confirm by Tests XIII and VI	Pale yellow or negative, cellulose acetate - propionate or butyrate. Confirm by Test VI and solubility
---	--



Table XI. Properties of Resins of Group E

Test No.	Property Sought	Polyvinyl Resins			Cellulose Esters			Poly-methyl Acrylate	Rosin Polybasic Acid
		Polyvinyl acetate	Copolymers of vinyl acetate and fumarates or maleates	High acetate polyvinyl alcohols	High acetyl cellulose acetate	Cellulose acetopropionate	Cellulose aceto-butyrate		
I	Solubility								
	95% ethanol	S	S-IG	S-P.S.G.	I	I	I	IG	S
	Acetone	S	S-SL.S.G.	IG	S-IG	S	S	S	S
	Ether	IG	IG-I	IG	I	I	I	IG	S
	Ethyl acetate	S	S-I	IG	S-I	S	S	S	S
	Dioxane	S	S-SL.S.G.	IG	S	S	S	S	S
	Pyridine	S	S-SL.S.G.	S	S-P.S.G.	S	S	S	S
	Acetic acid	S	S-IG	S-P.S.G.	P.S.G.	S	S	S	S
	Carbon tetrachloride	S	S-IG	S-P.S.G.	P.S.G.-I	I	IG	IG	IG
	Tetrachloroethane	S	S-SL.S.G.	S-I	S	S	S	S	P.S.G.
	Benzene	S	S-IG	IG	IG	I	IG	S	IG
II	Liebermann-Storch	Resin slowly turns green	Resin slowly turns green	Resin slowly turns green	Neg. or resin slightly brown	Neg.	Neg.	Resin slowly turns light brown	Dark orange
V	Carboxylic esters	Strong red violet	Strong red violet	Red-violet	Strong red violet	Strong red violet	Strong red violet	Neg.	Neg.
VI	Odor on ignition	Characteristic pungent	Ethereal + pungent	Pungent	Burning paper	Burning paper	Burning paper	Acrylate	Pine
	Char	Pos.	Pos.	Pos.	Pos.	Pos.	Pos.	Pos.	V.Sl.
	Fume	Heavy yel.	Heavy yel.	Heavy	Sl.	Cons.	Heavy white	Heavy white	Nil
	Distillate	V.Sl.	Sl.	V.Sl.	Nil	Sl.	V.Sl.	Much	Complete
XI	Formaldehyde	Red	Neg. or red to orange	Neg. to V. pale red	Red	Pale Yel.	Neg.	Neg.	Orange
XIII	Acetates	Purple to black	Blue to black	Purple	Red orange	Neg.	Neg.	Red orange	Neg.
	Acid No.	4.6	4.8	4	5	2	3	3	29
	Saponification No.	600	400-600	325-540	550	500	500	375	354
	Refractive index 20° C.	1.47	.....	1.47-1.51	1.48	1.47	1.49	1.49	...
	Specific gravity 20° C.	1.19	.....	1.2-1.26	1.27	1.29	1.20	1.2	1.15

Table XII. Separation of Group F

(Halogens, nitrogen, and sulfur absent. Saponification Nos. 120 to 325)

Soluble: Test solubility in ether

Soluble: Test solubility in acetone

Soluble: Do Test VI

or: Oily and  
acrolein, phenol  
and oil-modified  
alkyd. Confirm  
by Tests II and  
VII

Odor: Character-  
istic pungent,  
polyvinyl alco-  
hol, medium  
acetate type.  
Confirm: Soluble  
in water. Tests  
XI and XIII

Soluble: Test solubility in 95% ethanol

Partly soluble gel. Do Test VI

Odor: Formalde-  
hyde, regular  
alkyd. Confirm  
by Test VII

Odor: Acrolein  
and oily, oil-  
modified al-  
kyd. Confirm  
by Test VII

Soluble: Apply Test VI

Odor: Butalde-  
hyde, poly-  
vinyl butylal  
or coacetal.  
Confirm by  
Tests III, XI,  
and XIV

Odor: Character-  
istic pungent,  
polyvinyl al-  
cohol of low hy-  
drolysis. Con-  
firm by Tests  
III, XI, and  
XIV

Soluble: Apply Test VII

Negative: Butyl  
phenol formalde-  
hyde. Confirm  
by Tests I, II,  
VI, and XIV

Positive: Oil-  
modified al-  
kyd. Confirm  
by Tests II,  
VII, and XII

Table XIII. Properties of Resins in Group F

Test No.	Property Sought	Polyvinyl Acetals, 70% Hydrolysis	Polyvinyl Coacetal, BuH-AcH, 20% Ac, 12% OH	Polyvinyl Alcohols, Medium Acetate Type	Butyl Phenol Formaldehyde	Alkyds		
						Regular	Oil-modified	Phenol and oil-modified
I	Solubility							
	95% ethanol	S	S	I	P.S.G.	P.S.G.	P.S.G.	IG
	Acetone	S	S	I	S	S	S	I
	Ether	IG	IG	I	S	Sl.-P.S.G.	S-I	I
	Ethyl acetate	S	S	I	S	S	..	..
	Dioxane	S	S	I	S	S	..	..
	Pyridine	S	S	I	S	S	..	..
	Acetic acid	S	S	I	S	S	..	..
	Carbon tetrachloride	IG	IG	I	S	IG	I	I
	Tetrachloroethane	S	S	I	S	S	..	..
	Benzene	S	IG	I	S	IG	..	..
II	Liebermann-Storch	Resin orange to dark brown	Resin red to red brown to brown	Resin light brown to red brown	Violet to brown to red brown to muddy green	Usually neg. rarely brown	Brown to dark brown	Red to brown red to brown
III	Carbonate fusion							
	Odor	Pungent AcH	Pungent BuH	Pungent	Balsam + formaldehyde	Formaldehyde + musty	..	..
	Char	Pos.	Pos.	Pos.	Pos.	Pos.	..	..
	Fume	Heavy	Heavy	Heavy	Heavy	V.Sl.	..	..
V	Carboxylic esters	Red violet	Red violet	Red violet	Neg.	Violet	Violet	Red violet
VI	Odor on ignition	Pungent char-acteristic	Pungent BuH	Pungent char-acteristic	Phenol or cresol	Formaldehyde	Oily + acrolein	Oily + acrolein
	Char	Pos.	Pos.	Pos.	Pos.	Sl.	Nil	Pos.
	Fume	Heavy	Heavy	Heavy	Heavy brown	V.Sl.	Sl.	Sl.
	Distillate	Cons.	Nil	Nil	Cons.	Much	Complete	Cons.
VII	Phthalates	..	..	..	Neg. or weak pos.	Strong pos.	Pos.	Pos.
IX	Phenols	..	..	..	Red	Neg.	Neg.	Neg.
XI	Formaldehyde	Red	Orange	Red	V.Sl. violet	Neg.	Neg.	Neg.
XII	Xanthoproteic reaction	Neg.	Neg.	Neg.	Pos.	Neg.	Neg.	Neg.
XIII	Acetates	Red to green to blue	Slowly green	Violet to blue	Neg.	Neg.	Neg.	Neg.
XIV	Aldehydes	Red violet	Red violet	Neg.	Red violet	Neg.	Weakly pos.	Neg.
	Acid No.	6	8	4	2	20-50	25-50	10-30
	Saponification No.	255	140	120-325	125	150-250	140-225	150-250
	Refractive index 20° C.	1.46	....	1.51-1.55	1.66	1.57	....	....
	Specific gravity 20° C.	1.16	....	1.28-1.31	1.099	1.32	....	....



Table XIV. Separation of Group G

(Halogens, nitrogen, and sulfur absent. Saponification Nos. less than 120. Acetyl Nos. over 40)

## A. Test solubility in hot water

Soluble: Test solubility in benzene

Soluble: Polyethylene glycol waxes. Confirm: Soluble in dioxane, insoluble in ethyl acetate

Insoluble: Apply Test V

Positive: Polyvinyl alcohols, less than low acetate type. Confirm by Tests II and XIII  
Negative: Methyl cellulose. Confirm by Tests II, VI, and XIII

Insoluble: Test solubility in carbon tetrachloride (B)

## B. Solubility in carbon tetrachloride

Soluble: Test solubility in ether

Insoluble: Phenylphenol formaldehyde resin. Confirm by Tests VI and XII

Soluble: Do Test IX

Negative: Do Test XII

Negative: Ethyl cellulose. Confirm by Tests II and VI

Positive: Phenol indene cumarone. Confirm by Tests II and VI

Positive: Test solubility in 95% ethanol

Soluble: Substituted phenol formaldehyde. Confirm by Tests II, XII, and XIV

Insoluble: Butyl phenol formaldehyde. Confirm by Tests VI and XII

Insoluble: Test solubility in ethyl acetate (C)

## C. Solubility in ethyl acetate

Soluble: Do Test VI

Odor of burning paper, ethyl cellulose. Confirm by Tests II and XIV

Any other odor: Test solubility in ether

Soluble

Odor of Test VI cresol. Shows cresol acetaldehyde. Confirm by Tests II, IX, and XII

Odor of Test VI slight. Shows modified rosin. Confirm by Test II. Resin is dark red color

Insoluble: Test solubility in acetic acid (D)

Insoluble: Test solubility in acetic acid

Soluble: Do Test XI

Violet, polyvinyl formal. Saponification No. 20. Confirm by Tests II, V, and VI

Red, polyvinyl butylal. Acetyl No. about 250. Confirm by Tests II, V, and VI

Insoluble: Phenol resin. Confirm by Tests VI, XI, XII, and XIV

## D. Solubility in acetic acid

Soluble: Test solubility in benzene

Soluble: Test solubility in 95% alcohol

Soluble: Do Test XI

Brown: Polyvinyl acetal. Saponification No. 30. Confirm by Tests II, V, VI, and XIV

Red: Polyvinyl butylal. Acetyl No. about 150. Confirm by Tests II, V, VI, and XIV

Insoluble: Polyvinyl butylal. Acetyl No. about 100. Confirm by Tests II, V, VI, XI, and XIV

Insoluble: Polyvinyl formal. Saponification No. about 100. Confirm by Tests II, V, VI, and XI

Insoluble: Test solubility in 95% alcohol

Soluble: Rosin modified alkyl. Confirm by Tests II and VI

Insoluble: Benzyl cellulose. Confirm by Tests II, VI, and XIV

of concentrated sulfuric acid until a rich brown melt develops, cool, dilute with water, and render alkaline with 10% aqueous sodium hydroxide. Characteristic red color of phenolphthalein indicates presence of phenols. In cases where tarry matter obscures the color, dilute with water and confirm by discharging the color by acid. All phenolics tested, with the exception of an oil-modified one, gave a positive reaction with this test.

TEST IX. MILLON'S REAGENT FOR PHENOLIC RESINS. Prepare the reagent by dissolving 10 grams of mercury in 10 grams of fuming nitric acid without heating, then dilute with twice its volume of water, and filter off any precipitate, or allow it to settle.

Heat a small piece of resin with 1 cc. of clear reagent and boil about 2 minutes. A red color indicates phenols.

As the test is characteristic of the phenol group it is also given by some proteins. The absence of nitrogen will, however, direct the test to phenolic resins. A few phenolic resins fail to yield a positive test.

TEST X. CUMARONE-INDENE RESINS. This is a modified form of Ellis test (2, p. 1261 footnote; 3). With the latter it was found very difficult to decide whether the color was due to bromine or the resin. The modification gives a positive test with the usual cumarone resins but is negative with the low molecular weight polymers.

Dissolve 0.1 to 0.5 gram of resin in 10 cc. of chloroform, add 1 cc. of glacial acetic acid and 1 cc. of 10% bromine solution in chloroform, and let stand overnight. A red color indicates cumarone resins.

Do a blank at the same time.

Using 1 cc. of the highly colored solution, add about 1 to 2 cc. of 0.1 N sodium thiosulfate and shake vigorously. The blank will discharge to a light yellow color in the chloroform layer. A red color in the chloroform layer is evidence of the presence of high or medium molecular weight cumarone resins.

TEST XI. FORMALDEHYDE (6). Mix a small piece of resin and 2 cc. of 72% sulfuric acid (100 cc. of water and 150 cc. of concentrated sulfuric acid) plus a few crystals of chromotropic acid and heat by standing the test tube in a beaker of water at 60° to 70° C. for 10 minutes. Run a blank at the same time to avoid chance contamination from the laboratory air. A bright violet color indicates formaldehyde. Note the color after standing 1 hour at room temperature.

TEST XII. XANTHOPROTEIC REACTION. This test depends upon the presence of a phenyl group and is usually used to identify certain proteins which contain it. It is also shown by some oils and phenolic resins. It is sometimes useful as a confirmatory reaction.

Warm a small piece of resin with concentrated nitric acid for several minutes, cool, and add an excess of ammonium hydroxide.

In the presence of a phenyl group the nitric acid is yellow, changing to an orange on addition of the ammonium hydroxide.

TEST XIII. ACETATES (?). Add a 5% aqueous solution of lanthanum nitrate and 1 drop of 0.1 N iodine solution, followed by a drop of concentrated ammonium hydroxide, to a piece of the resin on a spot plate.

In the presence of acetates or propionates a brown or blue coloration quickly develops in the resin. This may occur before the ammonium hydroxide is added and indicates addition of iodine to the resin.

When in doubt, warm a piece of resin with a few drops of concentrated hydrochloric acid in 1 cc. of water for about 10 minutes and apply the test to about 0.5 cc. of the water, making sure sufficient ammonium hydroxide is added to render it ammoniacal.

TEST XIV. ALDEHYDES IN ACETALS (4). Heat a small piece of resin plus 1 cc. of reagent and 0.4 cc. of concentrated sulfuric acid on a steam bath for 2 to 3 minutes, then cool. Add a few drops of pure methanol and a layer of chloroform, then 0.5 cc. of concentrated hydrochloric acid, and shake the tube well. In the presence of aldehydes a red to purple color appears in the chloroform.

Reagent: 0.01 gram of azobenzene phenylhydrazine sulfonate in 100 cc. of distilled water.

## CLASSIFICATION ACCORDING TO TYPES AND GENERAL REACTIONS

Where the substance does not appear to give the confirmatory tests following its systematic separation by the above scheme, where, because of its history, distinction between only a few substances is required, this classification according to types with the reactions generally shown by them, will be found useful:

**Acrylate Resins.** Light-colored resins.  $n_D^{20}$  about 1.4. Specific gravity 1.2. Usually without filler. Soluble in acetone, esters, benzene; insoluble in  $CCl_4$ , 95% ethanol, ether. Test VI, sickly sweet odor of monomer, with practically complete distillation.

**Alkyd Resins.** Usually light color.  $n_D^{20}$  1.54–1.59. Specific gravity 1.1–1.4. Test II, usually brown. Test V, usually positive. Test VI, formaldehyde, oily or acrolein odor, considerable distillation. Test VII, usually positive. Usually insoluble in 95% ethanol, ether,  $CCl_4$ .

**Amino and Protein Resins.** Usually light-colored.  $n_D^{20}$  1.5. Specific gravity 1.1–1.35. All are insoluble in the solvents listed except melamine-formaldehyde, which is soluble in pyridine, acetic acid, and gelatin which is water-soluble.



Test No.	Property Sought	Polyvinyl Acetals					Polyvinyl Alcohol, Low Acetate Type	Polyethylene Glycol Waxes	Modified Rosin (Vinsol)
		Formaldehyde 75% hydrolyzed	Formaldehyde 95% hydrolyzed	Acetal 90% hydrolyzed	Resin turns orange to red brown	8% OH	Butylal 95% Hydrolyzed 12% OH	20% OH	
I	Solubility	IG	I	S	Resin turns orange to red brown	I.S.G.	S	S	S
	95% ethanol	P.S.G.S	I	IG	Resin turns orange to red brown	IG	IG	IG	S
	Acetone	I	I	S	Resin turns orange to red brown	S	S	S	S
	Ether	S	S	S	Resin turns orange to red brown	S	S	S	S
	Ethyl acetate	S	S	S	Resin turns orange to red brown	S	S	S	S
	Dioxane	S	S	S	Resin turns orange to red brown	S	S	S	S
	Pyridine	S	S	S	Resin turns orange to red brown	S	S	S	S
	Acetic acid	S	S	S	Resin turns orange to red brown	S	S	S	S
	Carbon tetrachloride	S	S	S	Resin turns orange to red brown	S	S	S	S
	Tetrachloroethane	S	S	S	Resin turns orange to red brown	S	S	S	S
II	Benzene	IG	I	S	Resin turns orange to red brown	IG	IG	IG	IG
	Xylene	..	..	..	Resin turns orange to red brown	..	..	..	..
	Hot water	I	I	I	Resin turns orange to red brown	I	I	I	I
	Liebermann-Storch	Resin slowly turns green	Resin slowly turns bistre	Resin turns orange to red brown	Resin turns orange to red brown	Resin turns orange to red brown	Resin turns orange to red brown	Resin turns orange to red brown	Resin turns orange to red brown
	Carboxylic esters	Red violet	Red violet	Red violet	Red violet	Red violet	Red violet	Red violet	Red violet
	Odor on ignition	Formaldehyde	Formaldehyde	Formaldehyde	Formaldehyde	Formaldehyde	Formaldehyde	Formaldehyde	Formaldehyde
	Char	Pos.	Pos.	Pos.	Pos.	Pos.	Pos.	Pos.	Pos.
	Fume	Cons.	Cons.	Cons.	Cons.	Cons.	Cons.	Cons.	Cons.
	Distillate	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
	Phthalates	..	..	..	..	..	..	..	..
VII	Phenolic	Deep violet	Deep violet	Deep violet	Deep violet	Deep violet	Deep violet	Deep violet	Deep violet
	Formaldehyde	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue
	Xanthoproteic reaction	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
	Aldehydes	4	4	4	4	4	4	4	4
	Acid No.	100	100	100	100	100	100	100	100
	Saponification No.	65	65	65	65	65	65	65	65
	Acetyl No.	..	..	..	..	..	..	..	..
	Refractive index 20° C.	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50
	Specific gravity 20° C.	1.23	1.23	1.23	1.23	1.23	1.23	1.23	1.23
	Specific gravity 20° C.	1.16	1.16	1.16	1.16	1.16	1.16	1.16	1.16

Test No.	Property Sought	Phenolic Resins					Substituted phenol formaldehyde	Phenolic resin	Phenol Indene Cumarone
		Resin Modified Alkyd	Methyl	Ethyl	Cellulose Ethers	Benzyl	Cresol-AcH	Butyl phenol formaldehyde	Phenyl phenol formaldehyde
I	Solubility	Red violet to brown	Resin slowly turns olive	Orange to black	Orange to black	Resin slowly turns orange to light brown	Red brown to orange	Violet to red to muddy green	Neg
	95% ethanol	S	I	P.S.G.S	P.S.G.S	I	S	P.S.G.	I
	Acetone	S	I	I-S	I-S	I	S	S	S
	Ether	S	I	S	S	I	S	S	S
	Ethyl acetate	S	IG	S	S	S	S	S	S
	Dioxane	S	IG	S	S	S	S	S	S
	Pyridine	S	IG	S	S	S	S	S	S
	Acetic acid	IG	I	I-S	I-S	I-G	S	S	S
	Carbon tetrachloride	IG	I	S	S	I	S	S	S
	Tetrachloroethane	S	IG	I-S	I-S	I	S	S	S
II	Benzene	..	..	..	..	..	..	..	..
	Xylene	..	..	..	..	..	..	..	..
	Hot water	I	I	I	I	I	I	I	I
	Liebermann-Storch	Red violet to brown	Resin slowly turns olive	Orange to black	Orange to black	Resin slowly turns orange to light brown	Red brown to orange	Violet to red to muddy green	Neg
	Carboxylic esters	V. pale violet	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
	Odor on ignition	Formaldehyde	Burning paper and pungent	Burning paper	Burning paper	Benzald. pungent	Cresol	Phenol or cresol	Pungent formaldehyde
	Char	Pos.	Pos.	Pos.	Pos.	Pos.	Pos.	Pos.	Pos.
	Fume	Cons. yel.	Cons.	Heavy white	Heavy white	Heavy yellow	Cons. brown	Heavy brown	Cons.
	Distillate	Weakly pos.	Nil	..	..	..	Cons.	Cons.	Cons.
	Phthalates	Neg.	..	..	..	..	Neg.	Neg.	Neg.
VII	Phenolic	Neg.	Neg.	Pale violet	Pale violet	Neg.	Red	Neg.	Neg.
	Formaldehyde	..	..	..	..	..	Pos.	Pos.	Pos.
	Xanthoproteic reaction	..	..	..	..	..	Pos.	Pos.	Pos.
	Aldehydes	7	3	Red	Red	Neg.	..	..	..
	Acid No.	90	120	65	65	100	49	2	50
	Saponification No.	1.54	1.54	1.47	1.47	1.47	370	295	270
	Acetyl No.	..	..	..	..	..	..	..	..
	Refractive index 20° C.	1.14	1.14	1.14	1.14	1.14	1.2	1.66	1.47-1.7
	Specific gravity 20° C.	1.14	1.14	1.14	1.14	1.14	1.099	1.21-1.27	1.21-1.27
	Specific gravity 20° C.	1.116	1.116	1.116	1.116	1.116	1.23	1.15-1.20	1.15-1.20



Table XVI. Separation of Group H

(Halogens, nitrogen, and sulfur absent. Saponification Nos. less than 120. Acetyl Nos. less than 40)

## A. Test solubility in ether

Soluble: Test solubility in 95% ethanol

Soluble: Test solubility in acetic acid

Soluble: Test for acid No.

Acid No. over 100, hydrogenated rosins. Confirm by Tests II and VI. Resin is brittle. Solid

Acid No. low, ethyl abietate. Confirm by Tests II and IV. Resin is viscous fluid

Insoluble: Do Test XII

Positive: Dihydro-methyl abietate. Confirm by Test VI. Only partly soluble in pyridine

Negative: Low-polymer cumarone oil. Confirm by Test VI. Soluble in pyridine

Insoluble: Test solubility in dioxane

Insoluble: Ter-pene resin. Confirm: Soluble in ligroin, insoluble in pyridine or acetone

Positive: Cumarone or polyindene resins. Confirm by Tests II and VI

Negative: Do Test II

Insoluble: Test solubility in acetic acid (I)

Negative or pale orange, hydrogenated cumarone indene resin. Confirm by Tests VI and XIV. Light-colored resin, insoluble in acetone and ligroin

Positive: Red purplish red, may change to green or brown, cumarone resin. Low m.p. semifluid resins are "soft" type. Hard solids "medium" type. Confirm by Tests VI and XIV. Mostly soluble in ligroin

## B. Solubility in acetic acid

Soluble: Poly-methyl methacrylate. Confirm by Test VI. Soluble in CCl<sub>4</sub>

Insoluble: Apply Test X

Positive: High m.p. cumarone resin. Confirm by Tests II and VI

Negative: Test solubility in dioxane

Soluble: Polystyrene. Confirm by Tests II and VI. Insoluble in acetone

Insoluble: Test solubility in benzene

Soluble: Resin usually dark and may contain some sulfur, isobutylene copolymer with diolefins. Confirm by Test VI. Soluble in CCl<sub>4</sub>

Insoluble: Resin light colored, polyisobutylene. Confirm by Test VI. Soluble in CCl<sub>4</sub>

Insoluble: Butadiene-styrene copolymer. Confirm by Tests II and VI. Insoluble in CCl<sub>4</sub>

Test III, NH<sub>3</sub> with burning hair odor characteristic of all except melamine-formaldehyde which gives a formaldehyde odor. Test VI, similar to Test III. Test XI, positive with urea-formaldehyde, melamine-formaldehyde, casein-formaldehyde. Negative with polyamide resin and gelatin. Test XII, positive with gelatin and casein-formaldehyde resins only.

**Cellulose Esters.** Usually light color.  $n_D^{20}$  1.47-1.51. Specific gravity 1.2-1.4. Nitrocellulose burns rapidly, other esters burn slowly; former yields Text IV. Test V, positive except for nitrocellulose. Test VI, odor of burning paper. Test XI, negative to red. Saponification No., 500 to 550.

**Cellulose Ethers.** Usually light color.  $n_D^{20}$  1.47. Specific gravity 1.10-1.25. Solubilities vary according to ethoxyl content, higher ethoxyl being more soluble. Methylcellulose soluble in hot water and insoluble in most other solvents. Test VI, odor of burning paper accompanied by benzaldehyde odor in case of benzyl cellulose. Test XIV, usually positive.

**Chlorinated Diphenyls.** Usually light color, vary from thin liquids to hard solids.  $n_D^{20}$  1.61-1.71. Specific gravity 1.34-1.95. Yield strong halogen test. Soluble in all solvents listed in Table II. Test VI, slight acrid odor, much distillation.

**Cumarone-Indene Resins.** Vary from light- to dark-colored liquid or solid resins.  $n_D^{20}$  1.6-1.66. Specific gravity 1.01-1.15.

Insoluble in 95% ethanol and acetic acid. Usually soluble in ether, acetone, esters, dioxane, or pyridine.

Test II, red color characteristic which may change to brown-violet, rarely to green. Test VI, indene-like odor and almost complete distillation. Test X, positive with higher molecular weight polymers. Hydrogenated cumarone resin insoluble in acetone and Test II weak orange; otherwise similar.

**Phenolic Resins.** Vary from light to dark resins, usually solid.  $n_D^{20}$  1.47-1.7. Specific gravity 1.1-1.27. Mostly soluble in pyridine, acetone, ether, tetrachloroethane, dioxane, and ethyl acetate, but usually insoluble in 95% ethanol. Heat-reacted form may be insoluble in all solvents.

Test II, may be negative but browns predominate. Test V, odor of phenol or formaldehyde with considerable distillation. Test IX, frequently positive. Test XII, positive. Test XIV, frequently positive.

**Resin Products.** Straw to highly colored liquids and solid.  $n_D^{20}$  1.52-1.61. Specific gravity 1.03-1.22.

Soluble in most solvents used in solubility test with the following exceptions: Polybasic acid insoluble in CCl<sub>4</sub>, benzene. Dihydro-methyl abietate, partially soluble in pyridine or acetic acid. Red colored modified resin (Vinsol) insoluble in CCl<sub>4</sub> or benzene.

Table XVII. Properties of Resins of Group H

Test No.	Property Sought	Cumarone Resins						
		Polymethyl Methacrylate	Terpene Resin	Polystyrene	Low polymer oil	Polyindene	Hydrogenated cumarone indene resin	High m.p. cumarone
I	Solubility	I	I	I	S	I	I	I
	95% ethanol	S	I	IG	S	S	I	S
	Acetone	I	S	IG	S	S	S	S-I
	Ether	S	Sl.S.G.	P.S.G.	S	S	S	S
	Ethyl acetate	S	Sl.S.G.	S	S	S	S	S
	Dioxane	S	Sl.S.G.	S	S	S	S	S
	Pyridine	S	Sl.S.G.	I	I	I	I	I
	Acetic acid	S	I	S	S	S	S	S-I
	Carbon tetrachloride	I	S	S	P.S	S	S + G	S
	Tetrachloroethane	S	S	S	S	S	S	P.S-S
	Benzene	S	S	S	S	S	S	....
	Ligroin	I	S	I	....	....	I	....
II	Liebermann-Storch	Neg.	Neg.	Neg.	Scarlet to red violet	Bright red	Light orange	Red to purplish red to brown
V	Carboxylic esters	Neg.	Neg.	Neg.	Neg.	....	Neg.	....
VI	Odor on ignition	Sickly sweet odor of monomer	Like coal gas	Styrene	Indene	Indene	Indene	Indene
X	Char	V.Sl.	Nil	Nil	Nil	Pos.	Nil	Sl.
	Fume	Sl. white	V.Sl.	Cons. white	Nil	Heavy yel.	Nil	Sl.
	Distillate	Complete	Complete	Complete	Complete	Much	Complete	Complete
XI	Cumarone resins	Neg.	Neg.	....	Neg.	Pos.	Neg.	Pos.
XII	Formaldehyde	Neg.	Neg.	Neg.	Orange	Neg.	Neg.	Neg.
XIII	Xanthoproteic reaction	....	....	....	Neg.	Neg.	Neg.	Neg.
XIV	Aldehyde	....	....	....	....	....	Red	....
	Acid No.	4	2	2	2	7	6	2
	Saponification No.	20	0	0	0	0	0	0
	Acetyl No.	0	0	0	0	0	0	0
	Refractive index 20° C.	1.49	...	1.59	1.60	1.6-1.66	...	1.6-1.66
	Specific gravity 20° C.	1.19	...	1.05	1.01	1.10	...	1.10



Test II, red to violet color. Test V. If any odor it is pine or balsamlike, resin distills without residue. Saponification number low except for hydrogenated rosins and the polybasic acid. Acid number over 100 only in case of the Vinsol resin. Acetyl number zero except for Vinsol resin.

**Rubber and Rubber Substitutes.** *Halogen Containing.* Chloroprene is black rubbery resin, characteristic odor, insoluble in all solvents. Rubber hydrochloride and chlorinated rubber light colored. Former insoluble in all except tetrachloroethane; latter soluble in ethyl acetate, dioxane, and pyridine. Cashew nut oil polymer is sticky black resin soluble in pyridine only.

*Butadiene Copolymers.* Black rubbery solids. Butadiene-styrene copolymer insoluble in all solvents. Butadiene-acrylonitrile copolymer insoluble in all solvents, contains nitrogen.

*Polyisobutylenes and Copolymers with Diolefins.* Light-colored solids to rubbery solids. Copolymers usually dark colored.

*Polysulfide Rubbers.* Contain much sulfur. Specific gravity 1.34. Soluble in dioxane, pyridine, tetrachloroethane. Test II, red violet changing to brown. Test VI, mercaptan odor, considerable distillate.

*Vulcanized Natural Rubber.* Contains sulfur.  $n_D^{20}$  1.52. Specific gravity 1.1–1.18. Insoluble in all solvents. Test VI, characteristic odor of burning rubber, no distillate.

**Sulfonamide Resins.** Light colored varying from soft viscous liquid to hard resins.  $n_D^{20}$  1.56–1.60. Specific gravity 1.31–1.36. Soluble in most solvents shown in Table VI, insoluble in  $CCl_4$ . Test XI, strong violet.

**Terpene Resin.** Light-colored solid. Soluble in ether,  $CCl_4$ , tetrachloroethane, benzene, ligroin. Insoluble in 95% ethanol, acetone, dioxane, ethyl acetate, acetic acid. Test VI, odor like coal gas, distills completely.

**Vinyl Resins.** *Halogen-containing.* Strong test for halogen. Very light-colored solids.  $n_D^{20}$  1.53–1.61. Specific gravity 1.2–1.75. Insoluble in ligroin and benzene. Test II, blue or green color slowly develops in resin.

*Polyvinyl Esters.* Colorless solids.  $n_D^{20}$  1.47. Specific gravity 1.19. Polyvinyl acetate soluble in all the solvents except ether and ligroin. Copolymers insoluble in ether and ligroin but may so be insoluble in the other solvents.

Test II, resin turns green; this is characteristic. Test V, red color. Test VI, characteristic odor, very slight distillate containing the acid usually acetic. Test XI, red to negative. Test XII, blue or purple to black. Saponification No. 400 to 600.

*Polyvinyl Alcohol-Acetate.* Light-colored resin.  $n_D^{20}$  1.47–1.55. Specific gravity, 1.2–1.33. Low and medium acetates insoluble in everything except water. High acetates soluble in pyridine. Test II, green to brown color. Test V, red violet. Test VI, pungent acidic odor, no distillate. Test XIII, violet to blue or purple. Acetyl number, low acetate type, 1080 to 1270, saponification No.: low acetate, 0 to 119; medium acetate, 120 to 325; high acetate, 325 to 540.

*Polyvinyl Acetals.* Light-colored resins.  $n_D^{20}$  1.46–1.50. Specific gravity 1.11–1.23. All insoluble in ether,  $CCl_4$ , ligroin. All soluble in dioxane, pyridine, tetrachloroethane, or acetic acid.

Test II, usually brown. Test V, red violet. Test VI, characteristic odor of aldehyde indicates type; little distillate. Test XI, violet with formals, red or brown with acetals, red with butyrols. Test XIV, usually red violet, formals may be negative.

*Polystyrene.* Light-colored resin.  $n_D^{20}$  1.59. Specific gravity 1.05. Soluble in dioxane, pyridine,  $CCl_4$ , tetrachloroethane, benzene. Insoluble in 95% ethanol, acetone, ether, acetic acid, ligroin. Test VI, odor of styrene, complete distillation. Saponification No. zero.

#### ACKNOWLEDGMENTS

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Table XVII. Properties of Resins of Group H (Continued)

Cumarone Resins (Cont'd)		Rosin Products			Synthetic Rubbers		
Medium m.p. cumarone	Soft cumarone	Ethyl abietate	Dihydro-methyl abietate	Hydrogenated rosin	Butadiene-styrene copolymer	Isobutylene copolymer with diolefins	Polyisobutylene
I	I	S	S	S	I	I	I
I-S	S	S	S	S	I	I	I
S	S	S	S	S	IG	I	Sl.S.G.
S	S	S	S	S	I	I	I
S	S	S	S	S	IG	I	I
S	S	S	P.S	S	I	I	I
I	I	S	P.S	S	IG	S	S
S	S	S	S	S	IG	S	S
S	S	S	S	S	IG	S	S
P.S-S	S	....	....	....	IG	P.S	....
Orange to brick red	Red to purplish red to green or brown	Red to violet to blue to black	Red to violet to purple to green	Red to violet to green to blue	Pale blue to gray green	Neg.	Neg.
....	....	Neg.	Neg.	Neg.	....	Neg.	....
Indene	Indene	Pine	Pine	Faint balsam	Slight styrene	V.Sl. aromatic	Like coal gas
Nil	Nil	Nil	Nil	Nil	Pos.	Sl.	Nil
V.Sl.	V.Sl.	Nil	Nil	Nil	Cons. white	Cons. white	V.Sl.
Complete	Complete	Complete	Complete	Complete	Considerable	Sl.	Nil
Pos. to neg.	Pos. to neg.	....	....	....	....	Neg.	....
Neg.	Neg. to weak violet	Red	Neg.	Neg.	....	Neg.	Pale violet
....	....	Pos.	Pos.	....	....	....	....
....	....	....	Neg.	Neg.	....	....	....
....	....	....	....	....	....	....	....
0	0	4	6	165	0	3	5
0	0	25	25	6	0	0	0
0	0	0	0	....	0	0	0
1.6–1.66	1.6–1.66	1.53	1.52	1.53	....	....	1.51
1.10	1.10	1.03	1.03	1.06	0.94	0.92	0.912



# Determination of Hydrocyanic Acid, Especially in Coke-Oven Gas

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The following method for rapid and accurate determination of hydrocyanic acid in coke-oven gas by a cyanogen bromide procedure, with a few changes should be of fairly general applicability in cyanide analysis. After absorption in potassium hydroxide in a special type of flask the sample is treated with an ammonium polysulfide solution to convert the cyanides to thiocyanates, thereby reducing the partial pressure of the free acid radical. The solution is then acidified at room temperature and carbon dioxide eliminated without appreciable loss of thiocyanate. Most of the gas is removed from the flask by at least partial evacuation, and, in the order named, the following reagents are added in excess: potassium bromide-bromate solution to convert thiocyanic acid to cyanogen bromide, phenol solution to eliminate excess bromine, and potassium iodide to reduce cyanogen bromide and substitute iodine. As this treatment is accomplished in an evacuated chamber, vapor pressure losses are zero. The liberated iodine is titrated with sodium thiosulfate solution. Studies were made which established conditions under which the action of the above reagents is substantially immediate. Laboratory time required for the analysis is about 15 minutes.

THE following procedure has been tried by the authors on many laboratory solutions and found satisfactory in contrast to other methods recommended for the determination of hydrocyanic acid by bromination followed by iodometric titration. It has been used successfully on coke-oven gas, where it is of particular value because of its wide range of applicability with respect to hydrocyanic acid concentrations. It also has a distinct advantage in that it integrates the analysis over a period of time as compared with the procedure of Seil (11) which employs an enlarged Tutwiler apparatus. This superiority is particularly desirable as the concentration of hydrocyanic acid in coke-oven gas often varies as much as perhaps 50% during a 30-minute interval. Incidentally, the Seil (11) method used on synthetic solutions of pure potassium cyanide standardized against silver nitrate gave results that were 16 to 20% high for quantities of hydrocyanic acid equivalent to a gas carrying 20 to 40 grains of hydrocyanic acid per 100 cu. feet, and a single test on a 2-grain gas showed 185% of the amount present. This appears to be due to the blank exhibited by dilute iodine solutions. (The Tutwiler iodine used in this test is approximately 0.013 *N*.) The Tutwiler procedure permits of no satisfactory compensation for this blank, nor can gas volume corrections be made.

The proposed method depends upon conversion of the cyanide or certain of its derivatives to cyanogen bromide, reduction of cyanogen bromide with potassium iodide, and titration of the liberated iodine with sodium thiosulfate solution. Several such procedures have been described in the literature. In the experience of the author and his associates, none of these have proved satisfactory, except perhaps under very restricted conditions. As the reactions involved have inherently great analytical advantages, a study of them was made which has resulted in the elimination of several sources of error, a widening of the scope of their application, and a considerable decrease in the time required for analysis. The laboratory time required for this analysis is about 10 to 15 minutes. Reproducibility obtained appears, in general, to be comparable to that involved in mechanical measurement of the standard solutions. This method was used to titrate a potassium thiocyanate solution carefully standardized by the Volhard procedure. The results varied from

the Volhard by 1.5 parts per thousand. For the quantities (20-ml. titrations) the probable mechanical error was 2 parts per thousand.

## SPECIAL APPARATUS AND SOLUTIONS REQUIRED

2 Shaw sulfur flasks (13).

Potassium hydroxide solution, 20%.

Ammonium polysulfide solution, which is prepared by taking 25 to 50 ml. of aqua ammonia, passing hydrogen sulfide through it at the rate of 2 to 3 bubbles per second, and adding an excess of micro sulfur to the solution. After about 15 minutes, the solution will be substantially saturated with sulfur and may be bottled for use. The ordinary analytical grade of yellow ammonium sulfide is not effective for this purpose.

Hydrochloric acid, concentrated reagent solution (1.18 sp. gr.). Bromide-bromate solution, 125 grams of potassium bromide and 25 grams of potassium bromate diluted to 1 liter with water, approximately normal with respect to potassium bromate. Phenol solution, approximately 5% phenol in water. Potassium iodide solution, about 50 grams per 100 ml. of solution. Thiosulfate solution, 0.1 *N* [0.01 *N* for a concentration of hydrocyanic acid below 1 grain per 100 cu. feet of gas (2.832 cu. meters) about 0.002% hydrocyanic acid]. Starch indicator solution.

## PROCEDURE I

(For concentrations of hydrocyanic acid above 5 grains per 100 cu. feet, 3.25 grams per 1000 cu. feet, about 0.01% hydrocyanic acid.)

Place 20 ml. of 20% potassium hydroxide solution in each of two Shaw sulfur flasks, connect in series for gas scrubbing, and scrub the gas at a rate of not more than 2.0 cu. feet per hour, with a meter at the end of the train. A 20 × 2.5 cm. (8 × 1 inch) test-tube tray may be used instead of the second sulfur flask. If the gas contains 10 grains of hydrocyanic acid per 100 cu. feet, a 1.0-cu. foot sample will give a final titration of about 5 ml. of 0.1 *N* thiosulfate solution. It is suggested that no more than 2.5 cu. feet of gas be taken as a sample because there is danger that a relatively large amount of carbon dioxide in the gas will destroy the caustic alkalinity and cause hydrocyanic acid to escape. After reading the meter, remove the train and combine the scrubbing solutions employing as little wash water as possible. Add 10 to 15 drops of the ammonium polysulfide solution, shake to mix, and let stand 2 minutes. In special instances where the volume of acid gas present is small, the polysulfide treatment may be omitted, in which case the flask containing the caustic solution is evacuated before subsequent acidification.

Remove the stopper and make the solution in the flask just acid by slowly adding concentrated hydrochloric acid, meanwhile swirling the flask to mix. If no extra alkali (such as ammonia) is present, this treatment will require about 10 ml. of acid. The end point is indicated by the disappearance of the yellow color of ammonium sulfide. Use a sufficient excess of hydrochloric acid to yield approximately normal acidity at the time the potassium iodide solution is subsequently added (8 ml. of concentrated hydrochloric acid per 100 ml. of solution are generally satisfactory). Avoid high local concentrations of acid in the solution. Cool the flask to room temperature if necessary, and evacuate. If the solution is saturated with carbon dioxide, this operation must be done carefully at first. The suggested procedure is to turn the channeled stopper in the flask to an open position and to attach a light suction to the outlet stopcock, so that a gentle bubbling takes place which can be maintained by progressively cutting down the size of the opening in the stopper plug.

Finally put on full suction, remove most of the air, and disconnect from the suction line. Through the adjustable vent in the funnel top of flask add increments of potassium bromide-bromate solution with shaking until an excess of about 2.0 ml. of the reagent is present after 5 minutes' standing. Usually 5 to 10 ml. are required. After a little experience the depth of bromine color in the sample will be a sufficient guide. A large excess of bromine should be avoided. Wash the funnel top with water to remove excess potassium bromide-bromate and pass a few



ops into the flask. Wash the tubes of the flask also with a little water after the addition of each reagent has been completed. Shake to mix and let the flask stand 2 to 3 minutes. Add about 1 ml. of the 5% phenol solution, wash into a flask with a few milliliters of water, and shake to mix, so as to eliminate all traces of yellow bromine color in the solution. Then add 4 ml. of potassium iodide solution, shake, and let stand 2 minutes. The line produced is a measure of the hydrocyanic acid in the sample. Vent the flask to the air, place a little distilled water in the funnel, and remove the stopper. Titrate the iodine with 0.1 *N* thiosulfate solution, adding starch indicator solution near the end. The titration, which is made in the "sulfur flask", is accomplished in two operations: initially swirling the flask to mix the thiosulfate and iodine, and then finishing after most of the iodine has disappeared by attaching a rubber tube to the inlet tube of the flask and blowing with the breath to mix during the addition of the last few milliliters of the thiosulfate.

CALCULATIONS. The reaction is based on the following equation:



then

$$\frac{\text{ml. of 0.1 } N \text{ thiosulfate} \times 2.084}{\text{cu. ft. of gas in sample (corrected)}} = \text{grains of HCN per 100 cu. ft. of gas (corrected)}$$

## PROCEDURE II

(For concentrations of hydrocyanic acid below 5 grains per 100 cu. feet, 3.25 grams per 1000 cu. feet, 0.01% hydrocyanic acid.)

Under the preceding procedure the size of gas sample, because of carbon dioxide present, is roughly limited to 2.5 cu. feet, which would require on titration only 6 ml. of 0.1 *N* solution on a gas containing 5 grains of hydrocyanic acid per 100 cu. feet. Much smaller titrations are undesirable and the use of 0.01 *N* solutions produces other well-known but not always realized complications. It has been found that, where ammonia is present in the gas in sufficient concentration, water can be used in place of potassium hydroxide solution as a scrubbing medium to yield sufficiently accurate results. For such a purpose the ammonia concentration of the gas is not permitted to drop much below 30 grains per 100 cu. feet throughout the test. The problem of conveniently adding such an amount of ammonia has required some consideration, as the volume of pure ammonia gas required (0.03 cu. feet per hour), is too small to be measured conveniently by a flowmeter; and aqua ammonia exhibits too high an initial partial pressure of ammonia to be used directly where it is necessary to scrub a large sample of gas, while with small gas samples an undesirably large amount of ammonia is left in the scrubbing solution. For all these reasons, procedure II, described below, is recommended for use where gas of very low hydrocyanic acid concentration is to be analyzed.

Set up two sulfur flasks and a 15 × 2.5 cm. (6 × 1 inch) test tube, each charged with 25 ml. of distilled water, as in procedure I except that a glass tee is placed in the gas sampling line just before the first sulfur flask and a sulfuric acid trap for ammonia is inserted before the meter. Connect three 15 × 2.5 cm. (6 × 1 inch) test tubes charged with 25 ml. each of aqua ammonia solution (28% reagent grade) in series, and, from a compressed source, pass air or other inert gas through a flow meter, through the aqua ammonia, and through the tee into the gas stream before the hydrocyanic acid scrubbers. Adjust the gas rate through the ammonia solution to approximately 10% of that of the gas passing through the hydrocyanic acid scrubbers, and at the conclusion of the test deduct the volume of the inert gas from the total gas reading to obtain the true volume of sample. At a total gas rate of 2 cu. feet per hour this combination will maintain the desired ammonia concentration in the gas throughout the passage of 10 cu. feet of sample without introducing excessive amounts of ammonia into the scrubbing solution. A 10 cu. feet sample will yield a titration of about 5 ml. of 0.1 *N* thiosulfate if the gas has a hydrocyanic acid concentration of 1 grain per 100 cu. feet. For larger samples (or room temperatures much above 60° C.) a greater volume of aqua ammonia should be used. For the analysis of the sample add the contents of the test-tube scrubber to the second sulfur flask, add ammonium polysulfide, and follow the directions in procedure I.

It is suggested that the contents of the two sulfur flasks be titrated separately with 0.1 *N* thiosulfate and the two titrations added together for the hydrocyanic acid calculation, though, if desired, the iodine solutions can be joined and titrated as one. The separate titration permits a comparison that throws light on the scrubbing efficiency and consequently on the ammonia

enrichment during scrubbing. The amount of thiosulfate required for titrating the solution in the first flask should be about four times that for the second flask. Under these conditions in this laboratory less than 2.5% error was indicated for analyses of a 1-grain gas. An additional water scrubbing unit will lower this error but will make the procedure more cumbersome. If it is desired to analyze gases having a hydrocyanic acid concentration below 1 grain per 100 cu. feet, it is suggested that 0.01 *N* thiosulfate solution be used, in which case the thiosulfate titration should be made at a temperature below 15° C. to avoid unreasonable blanks.

## FURTHER APPLICATIONS OF METHOD

It seemed that if this method is satisfactory for determining hydrocyanic acid in gas, it would be of utility in analyzing a wide variety of miscellaneous solutions containing hydrocyanic acid derivatives. This has indeed proved to be the case. The procedure has given reliable results in the standardization of potassium cyanide and potassium thiocyanate solutions, in the analysis of two types of wet-process gas purification liquors where it is a great time saver (as thiosulfate and chloride do not interfere), and in analyses of pure samples of cyanogen bromide.

The ammonium polysulfide treatment may be omitted in all cases where the gas liberated by acidification is insufficient to destroy the vacuum. In the analysis of alkaline cyanides, such as potassium cyanide, a small excess of fixed alkali should be added to the sample before evacuating the flask to prevent vapor loss.

Although the recorded reactions of cyanogen bromide offer several possibilities for its determination (1, 3, 5, 8, 9, 12), an iodometric method seemed most practical for routine laboratory use. Difficulties were encountered, however, with previously recommended procedures (2, 16, 17). The authors therefore decided to investigate the following factors: time required for oxidation and reduction, effects of pH during oxidation and reduction, influence of nature of acid present, and action of certain alkaline salts prior to reduction.

In studying the reduction phenomena, pure cyanogen bromide was weighed and transferred quickly to water in a volumetric flask. This stock solution was kept at 0° C. and fresh solutions were frequently prepared. Aliquot samples were taken for individual analyses. The pipets were filled by pressure instead of suction and the contents delivered below the surface of a fixed volume of water in an ordinary glass-stoppered Erlenmeyer flask. Known quantities of acid and potassium iodide were added, and the flask was quickly stoppered and let stand for a definite period of time. Finally the liberated iodine was determined with sodium thiosulfate.

Table I. Influence of Sodium Carbonate and Trisodium Phosphate on Cyanogen Bromide Analysis

Concentration of cyanogen bromide stock solution, 15.58 grams per liter (purity 97.5%). Aliquot for each determination, 10 ml. Concentration of sodium carbonate and trisodium phosphate solutions, 1.34 *N*. Volume of solution after acidification, 100 ml.; acidity, 1 *N*. Potassium iodide added, 4 ml. (0.5 gram per ml.). Concentration of sodium thiosulfate, 0.0822 *N*.

Alkaline Solution Used	Quantity Ml.	Reaction Time with Alkaline Solution	pH	Sodium Thiosulfate Ml.
				34.85 (Control)
Na <sub>2</sub> CO <sub>3</sub>	0.03	30 sec.	7.57	34.68
Na <sub>2</sub> CO <sub>3</sub>	0.03	30 min.	7.57 <sup>a</sup>	34.49
Na <sub>2</sub> CO <sub>3</sub>	10	30 sec.	9.96	29.81
Na <sub>2</sub> CO <sub>3</sub>	10	30 min.	9.96	7.67
Na <sub>2</sub> CO <sub>3</sub>	25	30 sec.	10.04	29.40
Na <sub>2</sub> CO <sub>3</sub>	25	30 sec.	10.04	29.04
Na <sub>3</sub> PO <sub>4</sub>	0.03	30 sec.	7.5 <sup>b</sup>	34.85
Na <sub>3</sub> PO <sub>4</sub>	0.03	30 min.	7.5 <sup>b</sup>	34.73
Na <sub>3</sub> PO <sub>4</sub>	10	30 sec.	10.90	24.43
Na <sub>3</sub> PO <sub>4</sub>	10	30 sec.	10.90	24.51
Na <sub>3</sub> PO <sub>4</sub>	10	30 min.	10.90	0.03
Na <sub>3</sub> PO <sub>4</sub>	25	30 sec.	...	18.77
Na <sub>3</sub> PO <sub>4</sub>	25	30 sec.	...	17.93
Na <sub>3</sub> PO <sub>4</sub>	25	30 min.	...	0.00

<sup>a</sup> During time interval solution became slightly acid.

<sup>b</sup> During time interval pH dropped to 6.1.

Inclusion of only one figure indicates reproducible result.



A study of the influence of time on the reduction of cyanogen bromide showed that while Schulek (10) specifies a 30-minute delay, actually the reduction is complete in less than 0.5 minute under the conditions employed. In order to learn the effect of acid concentration, nine determinations were made, in concentrations varying from 0.1 to 6.0 *N*. The results agreed within the experimental error. An investigation of the effect of the nature of the acid present was made by using several concentrations of acetic and phosphoric acids, including the concentrations used by Chattaway and Wadmore (3), Møller (6), and Schulek (10). The success of the reduction was found to be independent of the source of hydrogen ion, excluding, of course, oxidizing and reducing acids.

Buchanan (2), in accordance with Stevens and Blackett (15), suggests the addition of sodium carbonate to the sample, followed by slight acidification before conducting the analysis. Nardin (7), who originated the use of sodium carbonate, claims that "by previously neutralizing sulfuric acid present, more reliable and slightly higher results are obtained". This suggestion was investigated, and also, for purposes of comparison, the action of trisodium phosphate, with the results shown in Table I.

These findings indicate that, if the solution is made just alkaline with sodium phosphate no observable destruction occurs in 30 seconds, but with sodium carbonate a small, though measurable, quantity of cyanogen bromide is lost. In both cases increased amounts of alkaline solutions and longer reaction periods cause very marked decomposition. With carbonate there is observed an additional loss due to expulsion of cyanogen bromide with the carbon dioxide liberated upon acidification.

#### RECOMMENDED PROCEDURE FOR PURE CYANOGEN BROMIDE

Although, as the foregoing data indicate, the analysis of cyanogen bromide may be conducted under widely varying conditions, in this laboratory the following procedure has been adopted:

When a solution is to be analyzed, a suitable quantity of hydrochloric acid is placed in a glass-stoppered Erlenmeyer flask together with 4 ml. of the potassium iodide solution. An aliquot of the sample containing 0.1 to 0.2 gram of cyanogen bromide is then delivered just under the surface of the acidified solution and the flask is quickly stoppered. The solution is of such volume and concentration that after the addition of the sample there will be present 100 ml. of 1 *N* acid.

If a solid product is to be analyzed, individual samples of 0.1 to 0.2 gram are weighed in small glass-stoppered bottles, and dropped into 100 ml. of 1 *N* hydrochloric acid containing 4 ml. of potassium iodide solution.

In either case, after at least 30 seconds the liberated iodine is titrated in the customary manner with 0.1 *N* thiosulfate solution.

#### DISCUSSION OF METHOD

Several procedures appear in the literature for using this reaction as a measurement of hydrocyanic acid in gas. The authors and several associates have tried these methods with inexact results. As cyanogen bromide hydrolyzes in neutral or alkaline solution, previous analysts have added the potassium hydroxide gas-scrubbing solution slowly to an excess of strongly acidified bromine water. At least in fuel-gas analysis, large amounts of carbon dioxide are frequently present in the sample. Losses of cyanogen bromide in the released carbon dioxide inevitably occur, causing low results by the procedure mentioned. In the method here recommended, the hydrocyanic acid is converted to relatively nonvolatile thiocyanic acid by ammonium polysulfide, permitting release of carbon dioxide to the air upon acidification. The reaction to form potassium thiocyanate does not take place unless the polysulfide molecule contains more than 2 atoms of sulfur. If hydrogen sulfide is absent from the gas, 5 drops of the polysulfide solution are sufficient. If hydrogen sulfide is present, the sulfide formed appears to react with the polysulfide added, tending to reduce the sulfur ratio

below the 3 to 1 limitation. In dealing with unknown conditions, it is well to establish by trial and error the amount of polysulfide solution required. A very large excess is undesirable, but could not be readily avoided if the gas were actually scrubbed with polysulfide.

There is danger of hydrolysis of cyanogen bromide upon pouring an alkaline cyanide solution into acidified bromine water. This hydrolysis is extremely rapid in alkaline solution and there is the risk of a side reaction taking place at the interface of the two streams. A somewhat analogous condition exists if alkaline sulfide is run into acidified iodine, where very high results are occasioned by increased oxidation at the interface. The authors therefore, recommend that in all cases the sample be acidified before addition of bromine. If, for any reason, too high a concentration of acid is present after bromination during the analysis of a sample, the concentration should be diminished by dilution and not by addition of alkalis.

Potassium bromide-bromate solution is used instead of bromine water because it is more convenient to handle and the bromine value of the solution is constant.

The acidity of the solution is fixed at approximately *N* hydrochloric acid, a convenient strength to complete bromine and iodine oxidation reactions. Hydrochloric acid is preferred over sulfuric acid for this purpose. Very large excesses of acid are to be avoided.

If too large an excess of bromine is used, a large amount of tribromophenol will be thrown down as a precipitate, which has a tendency to adsorb the iodine subsequently formed in the solution and thus to give low results. A small amount of the precipitate does no harm, but preferably there should be very little. On the other hand, too small an excess of bromine, as indicated by only a faint yellow color in the solution, affords low results.

Eymann (4) says that excess phenol causes low results. The authors cannot confirm this statement. It seems probable that he attributed the action of tribromophenol precipitate to phenol itself. Skirrow (14) has pointed out that halogenated phenols tend to adsorb iodine, thereby giving rise to low results in such a titration.

In this reaction one hydrocyanic acid molecule is equivalent to two atoms of iodine, whereas in the titration of thiocyanate with silver and ferric alum the relation is 1 to 1 and in the titration of sodium cyanide with silver in alkaline solution, the ratio is 1 to 0.5. These ratios greatly favor the cyanogen bromide method, especially where low concentrations of cyanides exist in the sample.

In sampling, especially where the gas is water-saturated or contains ammonia, it is very important that the scrubbers be attached as close to the gas main as possible, for a very small amount of water condensate in the sampling line will remove an appreciable amount of hydrocyanic acid, especially in the presence of much ammonia.

Bromine converts both hydrocyanic acid and thiocyanic acid to cyanogen bromide. Cyanic acid is not converted to cyanogen bromide. Ferrocyanide is not converted to cyanogen bromide but is oxidized to ferricyanide, which in turn releases iodine, giving high results for hydrocyanic acid and thiocyanic acid present. This effect is eliminated if zinc sulfate is added in excess before the bromine. Heavy metal salts capable of oxidizing hydriodic acid must be removed as they will interfere with the analysis. These findings are important in analyzing miscellaneous solutions, but only the first two factors are likely to be of significance in gas analysis.

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# Photometric Determination of Phosphorus in Limestone

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A photometric method is described for the determination of phosphorus in limestone when present in amounts ranging from 0.002 to 0.4%  $P_2O_5$ . The sample is ignited to destroy organic matter, silica is removed by dehydration with perchloric acid, and phosphorus is determined in the filtrate by the phosphovanadomolybdate method. When applied to National Bureau of Standards samples of argillaceous limestone 1 and 1-a, containing 0.18 and 0.14%  $P_2O_5$ , respectively, results within 0.01% of the Bureau of Standards values were found. The effect of interfering elements and the use of a filter photometer are discussed.

THE accurate and rapid determination of phosphorus content of limestone used in the process is an important factor in carbide manufacture. Since the limestone usually contains 0.002 to 0.04% of phosphorus, great care must be exercised with gravimetric and volumetric methods of analysis if a high degree of accuracy and reproducibility is to be obtained. These methods are, however, too tedious and time-consuming for use in routine control.

Murray and Ashley (6) and Kitson and Mellon (2) used the colorimetric method suggested by Mission (5) to determine phosphorus in steel by converting the phosphorus to the yellow phosphovanadomolybdate complex. Willard and Center (1, 7) improved this colorimetric procedure by using perchloric acid instead of nitric acid to remove the silica, thus precluding high results from the formation of silicomolybdic acid. Koenig and Johnson (3) adapted the latter method to the determination of phosphorus in plants and in food materials.

The colorimetric determination of phosphorus as the phosphovanadomolybdate was found to be rapid and accurate when applied to the analysis of iron ores and plant ashes and offered promising possibilities for the analysis of limestones. If found applicable to this purpose, it was planned to adapt the method for use with a rugged, inexpensive photometer.

In this method the sample is calcined to destroy organic matter which, if not completely removed, imparts a slight color to the resultant solution; the calcium oxide is dissolved in perchloric acid and the resultant solution is fumed; and the yellow color of ammonium phosphovanadomolybdate develops upon the addition of ammonium vanadate and ammonium molybdate.

## APPARATUS

A Beckman Model D quartz spectrophotometer fitted with matched rectangular cells 1 cm. square.

Fisher AC Electrophotometer fitted with 425-m $\mu$  blue filter and 3-ml. cylindrical absorption cells.

## PRELIMINARY INVESTIGATION

Optimum conditions for the formation of the yellow phosphovanadomolybdate color have been investigated, and the amounts and composition of the necessary reagents have been

established (2, 3, 6, 7). Temperature was not regarded as critical so long as the color was formed at room temperature—that is, 20° to 30° C. (3, 6). Different investigators found various periods necessary for the development of the color. Willard and Center (7) stated that 4 minutes was sufficient, while others mentioned periods ranging up to 30 minutes (3, 6). Murray and Ashley (6) quoted Mission (5) as stating that after the color was developed it was stable for 14 days; whereas Koenig and Johnson (3) found slightly lower transmittance after 12 to 24 hours. Willard and Center (7) found that less than 13 ml. of perchloric acid per 100 ml. allowed the formation of a precipitate upon the addition of ammonium molybdate and that more than 13 ml. retarded full development of the color. Willard and Center (7) also investigated the effect of iron and found that a 30-m $\mu$  spectral band centered at 450 m $\mu$  obviated interference from ferric perchlorate.

Since it was desired to use a filter-type monochromator for photometric measurements, it was necessary to establish the relationship between the absorption characteristics of the filter, the possible iron salts present, and the ammonium phosphovanadomolybdate.

The absorption spectrum of the filter was determined and transmittance curves were made for solutions of ferric nitrate and ferric perchlorate, both of which may be considered present when the reagents specified (7) are used. Solutions were prepared by adding 100 mg. of  $Fe_2O_3$  as the corresponding salt to solutions containing 5 ml. of nitric acid and 17 ml. of perchloric acid and diluting each to 100 ml. A transmittance curve also was determined for a solution of ammonium phosphovanadomolybdate containing 0.1 mg. of phosphorus, prepared in the same manner as were solutions used for the calibration curve.

From data plotted in Figure 1 it can be seen that, although the absorption peak of the ammonium phosphovanadomolybdate is probably below 320 m $\mu$ , considerable absorption occurs in the spectral region covered by the blue filter. Figure 1 also shows that ferric perchlorate, even when present in as high an amount as 100 mg. of  $Fe_2O_3$ , absorbs only slightly in this region and that ferric nitrate exhibits more interference.

For this reason, it was decided to use perchloric acid instead of nitric acid in the preparation of the ammonium vanadate reagent. In the absence of nitrates it appeared that only minor difficulty would be experienced when working with solutions containing appreciable quantities of iron.

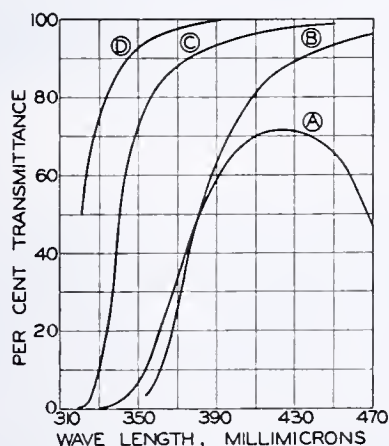


Figure 1. Transmittance Curves

- A. Fisher 425 blue filter  
 B. Ammonium phosphovanadomolybdate, 0.1 mg. of phosphorus in 100 ml. of solution  
 C. Ferric nitrate, 100 mg. of  $Fe_2O_3$  plus 5-ml. excess of nitric acid in 100 ml. of solution  
 D. Ferric perchlorate, 100 mg. of  $Fe_2O_3$  plus 17-ml. excess of perchloric acid in 100 ml. of solution

## REAGENTS

AMMONIUM VANADATE SOLUTION. Dissolve 2.35 grams of ammonium metavanadate in approximately 400 ml. of hot water; add 14 ml. of 72% perchloric acid, cool, and dilute to 1 liter.

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Table I. Effect of Excess Perchloric Acid upon Phosphovanadomolybdate Color

Phosphorus, Mg.	Per Cent Transmittance					
	HClO <sub>4</sub> , 13-Ml. Excess		HClO <sub>4</sub> , 15-Ml. Excess		HClO <sub>4</sub> , 17-Ml. Excess	
	After 30 min.	After 18 hours	After 30 min.	After 18 hours	After 30 min.	After 18 hours
0.0	94.5	94.5	94.0	94.0	94.5	94.5
0.2	72.8	72.4	72.9	72.3	73.0	72.6
0.4	58.2	57.7	58.5	57.8	59.0	58.6
0.6	48.0	48.0	48.6	48.5	49.2	48.8

**AMMONIUM MOLYBDATE SOLUTION.** Dissolve 100 grams of molybdic acid (85%) in a mixture of 300 ml. of water and 80 ml. of ammonium hydroxide. When dissolved, filter and boil filtrate 20 minutes; cool and dilute to 1 liter.

**STANDARD PHOSPHORUS SOLUTION.** Weigh out an amount of ammonium monohydrogen phosphate, the phosphorus content of which has been determined gravimetrically, equivalent to 0.1000 gram of phosphorus; dissolve in water and dilute to 1 liter. 1 ml.  $\approx$  0.1 mg. of phosphorus. (Theoretical amount of ammonium phosphate required, 0.4263 gram.)

#### FACTORS AFFECTING COLOR DEVELOPMENT

**ACID CONCENTRATION.** Using procedures similar to those recommended by Willard and Center (?), the effect of excess perchloric acid was investigated (Table I).

Table I shows that although greater excesses of perchloric acid do not allow the formation of as intense a color as does the 13-ml. excess, the color progression is insignificant after solutions have stood for 30 minutes. A 17-ml. excess of perchloric acid was chosen because the larger excess aided in the rapid dehydration of silica whenever large samples were necessary.

**EFFECTS OF IRON AND CALCIUM.** Iron in the concentration usually encountered in limestone causes little interference. Calcium salts have no effect on color development.

#### PROCEDURE

**CALIBRATION CURVE.** Transfer aliquots of the standard phosphorus solution to 100-ml. volumetric flasks containing 17 ml. of 72% perchloric acid. Add 10 ml. of ammonium vanadate solution, dilute to 75 ml., and cool to about 25° C. Add 7.5 ml. of ammonium molybdate solution, swirling the contents of the flask meanwhile to prevent precipitation. Dilute to the mark, mix thoroughly, and allow to stand for 30 minutes. Determine the percentage transmittance using a Fisher Electrophotometer with a 425-m $\mu$  blue filter. Plot the results on semi-log paper.

**ANALYTICAL METHOD.** Ignite a sample of limestone (depending upon the phosphorus content) in a porcelain crucible for 30 minutes at 900° C. If a large amount of organic matter is present, ignite the sample for 15 minutes at 500° C. before igniting at the higher temperature. Transfer the ignited residue to a 150-ml. beaker, add 20 ml. of water, and dissolve the calcium hydroxide with 72% perchloric acid, in the following proportions:

0.5 gram:18 ml.	2.0 grams:20 ml.
1.0 gram:19 ml.	5.0 grams:25 ml.

Evaporate on a hot plate until fumes of perchloric acid are evolved; cover with a watch glass and continue the fuming for 5 minutes to dehydrate the silica. Cool to below 100° C., and add 10 ml. of ammonium vanadate solution. Rinse the watch glass and sides of the beaker with a jet of water, limiting the washings to 15 ml. Mix the solution, cool to room temperature and filter through a Whatman 41-H paper into a 100-ml. volumetric flask. Wash the beaker and paper three times, restricting the volume to less than 90 ml. Cool the solution to about 25° C., while keeping the solution continuously agitated by shaking; add 7.5 ml. of ammonium molybdate solution and dilute to the mark. Mix the contents of the flask thoroughly and allow to stand 30 minutes. Determine the percentage transmittance, using a Fisher electrophotometer with a 425-m $\mu$  glass filter. Calculate the percentage phosphorus from the number of milligrams of phosphorus found on the calibration curve.

#### APPLICATION TO STANDARD SAMPLES

Samples of Bureau of Standards argillaceous limestone and dolomite were analyzed by the photometric method. The

values obtained by the Bureau of Standards, the mean of values by cooperating analysts, and the values by the photometric procedure are given in Table II. Up to this time the term "phosphorus" has been used, since it is customary to report the P<sub>2</sub>O<sub>5</sub> present in limestone used for carbide manufacture in terms of the element. The Bureau of Standards certificates of analysis are on the P<sub>2</sub>O<sub>5</sub> basis; therefore results on these samples are reported as the pentoxide.

#### PRECISION AND ACCURACY

**PRECISION.** The results reported in Table II are given as the nearest hundredth per cent P<sub>2</sub>O<sub>5</sub>. In Table III, the original values are given to show the precision of the method.

From the results in Table III the average deviation from the mean was found to be 0.0037% P<sub>2</sub>O<sub>5</sub>. The probable error of a single determination using the method of least squares was found to be 0.0028% P<sub>2</sub>O<sub>5</sub>.

**ACCURACY.** A comparison of the results in Table II shows that the results by the photometric method are within 0.01% P<sub>2</sub>O<sub>5</sub> of the values reported by the Bureau of Standards when applied to samples containing 0.14 and 0.18% P<sub>2</sub>O<sub>5</sub>. A maximum deviation of 0.001% P<sub>2</sub>O<sub>5</sub> from the Bureau of Standards value was obtained on a sample of dolomite reported to contain 0.002% of P<sub>2</sub>O<sub>5</sub>.

Table II. Analysis of Bureau of Standards Samples

Sample	Bureau of Standards Value P <sub>2</sub> O <sub>5</sub> %	Average of Cooperating Analysts P <sub>2</sub> O <sub>5</sub> %	Photometric P <sub>2</sub> O <sub>5</sub> %	Deviation from Standard %
Argillaceous limestone, No. 1	0.18	0.18 <sup>a</sup>	0.19 <sup>b</sup>	+0.01
Argillaceous limestone, No. 1-a	0.14	0.15 <sup>c</sup>	0.14 <sup>d</sup>	0.00
Dolomite, No. 88	0.002	0.003 <sup>e</sup>	0.002 <sup>f</sup>	0.000

<sup>a</sup> Average of two values of 0.18 and 0.18.

<sup>b</sup> Average of three values ranging from 0.18 to 0.19.

<sup>c</sup> Average of ten values ranging from 0.108 to 0.18.

<sup>d</sup> Average of seven values, all 0.14.

<sup>e</sup> Average of two values of 0.002 and 0.004.

<sup>f</sup> Average of seven values ranging from 0.002 to 0.003.

Table III. Precision of Method

Sample 1-a	P <sub>2</sub> O <sub>5</sub> Found, %	Deviation from Mean, %
	0.135	-0.005
	0.143	+0.003
	0.137	-0.003
	0.142	+0.002
	0.144	+0.004
	0.135	-0.005
	0.144	+0.004
Av.	0.140	

#### SUMMARY

A photometric method is described for the determination of phosphorus (0.002 to 0.4% P<sub>2</sub>O<sub>5</sub>) in limestone. The method is based upon the phosphovanadomolybdate color reaction, and may be used with a simple filter photometer. Calcium and iron salts, in the quantities encountered in limestone, cause no interference, and the organic matter is destroyed by a preliminary calcination.

Results obtained by the method have an accuracy and reproducibility adequate for the evaluation of limestone used for carbide manufacture. The procedure effects a great saving in time and reagents.

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# Determination of Tin by a Modified Iodometric Method

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An accurate method for the determination of tin is proposed which avoids the usual sources of error encountered in precipitating tin as metastannic acid and those encountered in the reduction-oxidation procedure. The metal is dissolved in acid and, when necessary, protected with the use of ammonia and aluminum hydroxide, reduced with nickel, and titrated with standard iodine solution under a blanket of carbon dioxide.

It is generally accepted that the best method for determining tin is based on the reduction of the tin to its bivalent state with a metal, and subsequent oxidation with a standard iodine solution. Details of procedure vary widely and many difficulties are encountered that seriously affect the usefulness of the method. Sources of error encountered are:

- 1. Incomplete precipitation of metastannic acid in making separations from copper
- 2. Loss of precipitated metastannic acid in filtering
- 3. Failure to obtain a satisfactory titration end point
- 4. Incomplete reduction of the material
- 5. Failure to prevent oxidation of stannous chloride by contact with air of the reduced solution

It is necessary to ensure the complete reduction of the tin to its bivalent state and to prevent its reoxidation by air. The former is not difficult and can be accomplished by the use of several metals. "Success in the latter depends on the maintenance of a nonoxidizing atmosphere during the entire operation, which is possible through the use of such expedients as the Bunsen burner or the use of a few grams of sodium carbonate" (2). The precipitation of tin by the usual nitric acid procedure is known to be incomplete at times (4) because metastannic acid often fails to coagulate sufficiently to be completely retained upon filtration. By the proposed method, precipitation is complete without filtration loss.

A series of determinations was made in order to select a metal to be employed as the reducing agent. The metals used were:

iron (3), lead (5), antimony (6), and nickel (1). All these metals are capable of effecting the reduction of tin to the bivalent state. Nickel, alone, gave a very sharp end point. It is unnecessary to remove the undissolved nickel, thus eliminating from the analysis a step which is a troublesome source of error.

No claim to originality is made as regards the individual details of the procedure finally adopted. However, because the procedure outlined differs in some detail from any of those published and the results obtained are precise and accurate, it is believed that the following method is to be preferred.

## REAGENTS REQUIRED

**STANDARD TIN SOLUTION.** Dissolve 5 grams of pure tin in 100 ml. of concentrated hydrochloric acid and dilute to 1 liter in a volumetric flask.

**STANDARD IODINE SOLUTION.** Dissolve approximately 11 grams of iodine in about 100 ml. of distilled water containing 20 grams of potassium iodide. Dilute to 1 liter in a volumetric flask and standardize against the standard tin solution after reduction of 25 ml. of the tin solution by the method given below.

**STARCH SOLUTION.** Add a thin paste made of 5 grams of soluble starch, 10 grams of sodium bicarbonate, and water to about 300 ml. of boiling water. Boil for 1 minute with stirring. Cool rapidly and dilute to 1 liter.

**PURE NICKEL** (shot or strip).

## PREPARATION OF SAMPLES FOR REDUCTION

**BRASS, BRONZE, AND COPPER BEARING MATERIALS** (over 2% copper). Weigh 1 to 10 grams of material to give a tin content between 0.1 and 0.2 gram into 300-ml. Erlenmeyer flasks, add 10 to 30 ml. of nitric acid (1 to 1), and heat gently until the material is completely disintegrated. Boil until oxides of nitrogen are expelled, dilute to about 100 ml. with water, and add 3 to 5 ml. of 10% aluminum nitrate solution. Add ammonium hydroxide to the blue copper complex color, then 10 ml. in excess. Heat to boiling and filter through hard paper (Whatman No. 42 or comparable). Wash twice with 5% ammonium nitrate solution. Place the filter paper containing the precipitate in the original flask. Add 10 ml. of concentrated sulfuric acid, 5 ml. of concentrated perchloric acid, and a few drops of concentrated nitric acid. Heat gently, adding nitric acid dropwise as required to prevent darkening of the solution. Evaporate to sulfur trioxide fumes. Cool first in air, then in water, and proceed as directed below.

**SOLDER BEARING METAL, etc.** (less than 2% copper). Weigh the sample to contain between 0.1 and 0.2 gram of tin into 300-ml. Erlenmeyer flask. Add 10 ml. of concentrated sulfuric acid and about 5 grams of potassium sulfate, and heat until the material is completely dissolved or until lead sulfate, if present, turns white. Cool first in air, then in water, and proceed as directed below.

## METHOD

Carefully dilute with water to about 100 ml., add 75 ml. of concentrated hydrochloric acid and 10 grams of nickel shot (10-mesh), and connect flask with a tube, one end of which extends below the surface of the beaker of water. Boil gently for 30 minutes, transferring the end of the outlet tube to a beaker of sodium bicarbonate solution (10%) several minutes before the end of the period.

Keeping the end of the tube below the surface of the sodium bicarbonate solution (Figure 1), place the flask in a suitable container of cold water. Allow to stand until cold. Remove the rubber stopper and, as rapidly as possible, introduce a small piece of dry ice (solid carbon dioxide). Then add more dry ice in sufficient quantity to keep the solution very cold (below 10° C.) and blanketed with carbon dioxide gas. (If solid carbon dioxide is not available, pellets of sodium bicarbonate may be substituted and will give satisfactory results if the solution is cooled by the use of ice.) Add 5 ml. of starch solution and titrate at once against the standardized iodine solution to a permanent blue end point. Calculate the percentage of tin in the sample.



Figure 1. Apparatus



Table I. Determination of Tin<sup>a</sup>

Bureau of Standards No.	Material	Tin Present	Tin Found	Deviation
		%	%	%
62	Manganese bronze	0.82	0.82	0.00
37	Sheet brass	1.013	1.012	0.001
53	Lead-base metal	10.91	10.91	0.00
127	Solder	34.88	34.87	0.01
52	Cast bronze	7.90	7.90	0.00
63	Phosphor bronze	9.91	9.91	0.00
54a	Tin-base metal	88.61	88.61	0.00

<sup>a</sup> Figures represent average of six determinations of each sample.

#### DESCRIPTION OF APPARATUS

The apparatus consists of Erlenmeyer flasks (300-ml.), stoppered with one-hole rubber stoppers. These are provided with bent glass and rubber tubing that extends to the bottom of the beakers containing, as they are used, water and bicarbonate of soda. The flask rests upon an electric heater that has a regulator to permit high, medium, or low heat adjustment. A ring stand,

the base of which fits snugly under the heater, is provided with cross supports for the bent glass tubing. At the sides of the heater are two 80-ml. beakers partly filled with ice and water to facilitate rapid cooling.

Table I shows results obtained on National Bureau of Standards samples of varying tin content.

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THE views presented in this article are those of the writers and are not to be construed as the official views of the Navy Department.

## Purification of Solvents for Absorption Spectroscopy An Adsorption Method

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A simple, rapid method for removing ultraviolet-absorbing impurities from hydrocarbon solvents by selective adsorption on silica gel columns is described. Solvents suitable for use in absorption spectrum measurements have been prepared by this method from commercial samples of cyclohexane, *n*-heptane, iso-octane, Skellysolve-B, and Skellysolve-F. In general, hydrocarbon solvents, both synthetic and commercial, which have been subjected to exhaustive chemical and physical purification have been noticeably improved by this adsorptive treatment. The advantages of the adsorption method over the usual methods are speed, simplicity of technique, and high yield of purified solvent.

IN RECENT years the advantages of liquid hydrocarbons over polar liquids as ultraviolet absorption solvents have gained recognition (1). The need for the use of pure hydrocarbons such as synthetic *n*-heptane, cyclohexane, and iso-octane instead of the commercially available petroleum fractions, which are hydrocarbon mixtures, has been demonstrated in specific examples (2, 5, 11). In this connection the purification of liquid hydrocarbons has been investigated.

The present methods for the preparation of hydrocarbons for use as solvents in absorption spectroscopy (3, 8, 9, 11) are as a rule long and cumbersome and result in poor yields. Usually the final products still contain significant amounts of impurities, probably aromatic and unsaturated compounds, which absorb radiations in the ultraviolet region.

The present paper describes a simple method for direct purification of commercial hydrocarbon solvents based on selective adsorption of the impurities by means of a suitable adsorbent. The adsorption procedure was suggested by the work of Mair, White, and others (4, 7, 10) who, in connection with an investigation of the composition of petroleum distillates at the National Bureau of Standards, showed that aromatic hydrocarbons can be separated from naphthenic and paraffin hydrocarbons by adsorption on silica gel.

The results which the authors obtained demonstrated the effectiveness of silica gel for purifying not only synthetic heptane, cyclohexane, and iso-octane for absorption spectroscopy but also petroleum ether fractions (Skellysolves), if desired. In general, both synthetic and commercial hydrocarbons, even after they have been subjected to exhaustive chemical and physical purification, have been noticeably improved in ultraviolet transparency by adsorptive treatment.

#### APPARATUS AND PROCEDURE

The adsorption apparatus used consists of a glass tube, 120 in. length and 38 to 40 mm. in diameter, constricted at the lower end. A small plug of glass wool is placed on a perforated porcelain disk at the constricted end of the column and about 100 grams of silica gel, Davco 659528-2000 (manufactured by Davison Chemical Corporation, Baltimore, Md.), are introduced with the aid of a powder funnel in batches of about 100 grams. The tube is tapped occasionally to ensure good settling of the adsorbent. Another plug of glass wool is placed on top of the column to prevent agitation of the adsorbent by the pouring of the solvent to be purified.

The solvent is added to the column from a 2-liter separatory funnel, care being taken not to allow the top of the column to run dry before all the solvent has been added. The solvent is allowed to percolate through the column and the percolate is collected in the same manner as the successive fractions of a distillation. The first fraction is in all cases the purest sample. Successive fractions are acceptable until the adsorbent has become saturated with respect to the impurities. A test spectrogram is made to ascertain the extent of purification in the successive fractions. Usually a single passage of the liquid through the column suffices to produce a satisfactory ultraviolet-transmittance solvent, and a yield of about 90 to 95% of the original liquid is obtained. A simple distillation may be used to remove any remaining adsorbent.

Various experiments were performed to determine the maximum amount of liquid which could be purified with a given quantity of silica gel and other adsorbents. However, no definite value was found to vary according to the activity of the particular adsorbent, and even more widely with the amount of impurities to be removed from different solvents or the same type of solvent.



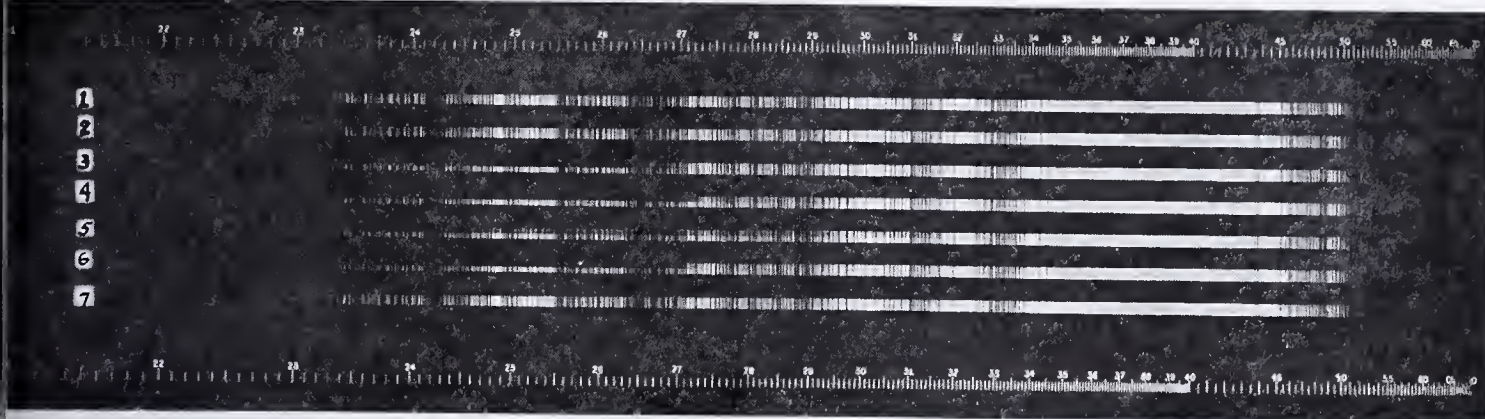


Figure 1. Comparative Spectrograms of Hydrocarbons

Upper portion, before purification; lower portion, after purification

1. Water, doubly distilled
2. Iso-octane
3. Skellysolve-F
4. Skellysolve-B
5. n-Heptane
6. Cyclohexane
7. Water, double distilled

Table I. Optical Density of Hydrocarbons before and after Purification by Silica Gel Adsorption Method (4-Cm. Cell)

Hydrocarbon	Purification	Wave Length				
		2300 Å.	2400 Å.	2500 Å.	2600 Å.	2700 Å.
Hexane, Alkyl Chemical & Co.	Before	∞ <sup>a</sup>	∞	∞	∞	∞
	After	0.10	0.00	0.00	0.00	0.00
Hexane, Dow Chemical Co.	Before	∞	∞	∞	∞	0.49
	After	0.24	0.01	0.00	0.00	0.00
Hexane, Du Pont Co.	Before	∞	∞	∞	∞	∞
	After	0.12	0.00	0.00	0.00	0.00
Hexane, Eastman Kodak Co.	Before	∞	∞	∞	∞	∞
	After	0.52	0.10	0.00	0.00	0.00
Hexane, Practical	Before	∞	0.61	0.84	0.72	0.50
	After	0.11	0.08	0.00	0.05	0.00
Heptane <sup>b</sup> , Eastman Kodak Co.	Before	∞	∞	1.34	0.13	0.16
	After	0.05	0.02	0.00	0.06	0.00
Heptane <sup>c</sup> , Rohm & Haas	Before	0.29	0.08	0.10	0.17	0.28
	After	0.00	0.00	0.00	0.00	0.16
Skellysolve-B <sup>d</sup>	Before	∞	∞	∞	∞	∞
	After	0.07	0.06	0.06	0.02	0.00
Skellysolve-F <sup>e</sup>	Before	0.30	0.98	1.05	0.98	0.05
	After	0.00	0.00	0.00	0.04	0.00

<sup>a</sup> ∞ represents insufficient transmission through 4-cm. layer to blacken graphic plate.

<sup>b</sup> 2,4-Trimethylpentane, b.p. 98-99° C.

<sup>c</sup> 2,4-Trimethylpentane, Bureau of Standards certified grade.

<sup>d</sup> Petroleum ether, b.p. 60-71° C. (A.S.T.M.).

<sup>e</sup> Petroleum ether, b.p. 30-60° C. (A.S.T.M.).

different commercial sources. Experience has shown that 100 gms of silica gel will purify 1 to 1.5 liters of cyclohexane, 1 liter of n-heptane, at least 4 liters of iso-octane, 0.5 to 1 liter of Skellysolve-B, and 2 to 2.5 liters of Skellysolve-F.

Other adsorbents such as alumina, magnesium oxide, various clays, and carbons were tried. Effective improvement of solvent was accomplished by the use of decolorizing carbons (Darco, Eastman), and activated charcoal, U.S.P. (Fisher).

Activated silica gel of the type described is an efficient and readily available adsorbent; it was chosen especially because samples of this material are easily prepared and allow the solvent to pass through rapidly. This adsorbent can be readily regenerated for most purposes by washing thoroughly with water and heating in a stream of air at 325° C. for 24 hours (4).

Attempts were made to purify the hydrocarbons by adding the adsorbent directly to the solvent to form a slurry, and subsequently removing the adsorbent by filtration. The concentration of aromatics, as shown by spectrograms, was noticeably decreased. However, continuation of the slurry procedure showed that complete removal of the ultraviolet-absorbing impurities could not be achieved, if at all, only after preparing a large number of slurries with recharges of fresh adsorbent, thus requiring a high ratio of adsorbent to solvent purified.

Figure 1 shows absorption spectra of a series of different hydrocarbons before and after a single passage through a silica gel ad-

sorbing column. It illustrates the effectiveness with which the ultraviolet-absorbing impurities are removed from various hydrocarbons.

The spectrograms were obtained by use of a Bausch & Lomb medium quartz spectrograph and a Hilger Spekker photometer. The absorption cell which usually contains the solvent was filled with the hydrocarbon as obtained commercially, and the cell which usually contains the solution was filled with the liquid obtained from a single passage through the silica gel. The photometer drum was set to provide equal apertures and consequently to permit equal amounts of light to strike each cell. The source of radiation was a high-voltage alternating current arc, using Therapeutic Carbon "B" rods, which provides a many-lined spectrum of iron superimposed on a continuous background of temperature radiation from the carbon (6). A corresponding quantitative representation of the improvement observed in the transmitting properties of these liquids throughout the spectral region 2300 to 2700 Å. is shown in Table I. The optical densities were obtained from measurements made with a Leeds & Northrup recording densitometer of spectrograms obtained by photographing each liquid, before and after purification, in 4-cm. cells, each against a matched empty cell representing 100% transmission.

## ACKNOWLEDGMENTS

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# Sulfuric Acid Extraction in Hydrocarbon Type Analysis

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The extrapolation method based on acid extraction is suggested as a means of routine and control analysis of paraffin-naphthene-aromatic or paraffin-naphthene-aromatic-olefin mixtures low in olefin concentration, boiling in the kerosene-to-gas-oil range. The procedure described is suitable for hydrocarbon mixtures containing 20% or less of aromatics and olefins. Where applicable, the extrapolation method gives a direct measure of saturate content and properties. This is sufficient, in conjunction with a molecular weight determination, to establish the paraffin-naphthene ratio. At the same time a measure of olefin plus aromatic content is obtained and approximations as to the character of the unsaturates (olefins and aromatics) can be made.

TYPE analysis of hydrocarbons boiling above the gasoline range has recently assumed considerable importance, in keeping with the development of new petroleum cracking processes and high-solvency petroleum solvents (10). This paper suggests sulfuric acid extraction for routine type analysis of medium boiling range hydrocarbon solvents, refinery cracking charge stocks, and the like, which contain small to moderate proportions of aromatics and olefins, not exceeding 20% of the sample.

A comprehensive method of type analysis based on combustion for hydrogen content is available from the work of Deanesly and Carleton (3, 4). Organic combustion for hydrogen, however, is rather tedious for a refinery laboratory and there would appear to be considerable incentive to avoiding this step if possible without too great a sacrifice of accuracy (12). With the object of substituting some simpler procedure for routine examinations, the well-known use of sulfuric acid as an extracting agent has been considered in this laboratory.

Sulfuric acid extraction has been advocated from time to time by a number of investigators (6, 7, 9, 11, 15) as a means of separating unsaturated from saturated hydrocarbons in type analysis of mixtures. Use of the acid as an analytical tool has been objected to (6, 13), however, on the generally valid grounds of inaccuracy due to incomplete removal of unsaturates, solubility of saturates in the acid extract, or chemical attack by the acid (18).

A stepwise extraction procedure and graphical interpretation of the extraction data have been investigated by Fisher and Eisner (6) for hydrocarbon type analysis. In Fisher and Eisner's method, acid ranging in concentration from 75 to 98% by weight was employed in a series of about ten successive treatments, at a constant ratio of 3 volumes of acid to 1 volume of oil. Graphs relating the physical constants and volume of unsulfonated oil were obtained which showed density and refractive index maxima in the earlier extraction stages, followed by progressive decreases to approximately constant values in the later stages. The maxima were interpreted as corresponding to complete olefin removal and incipient aromatic extraction, and the approximately constant later values of density and refractive index were interpreted as representing complete aromatic extraction and incipient removal of saturates.

Earlier work indicated that a two- or three-step extraction procedure, utilizing strong acid and varying the volume of acid, might yield linear volume-physical constant data and thereby simplify analysis.

In the experiments described below, it was found that acid sufficiently strong to remove aromatic compounds completely from a kerosene cut also reacted with the saturates present. Nevertheless, the action of the acid on the saturates could be allowed for by

using several different ratios of acid to oil and extrapolating to hypothetical zero acid to oil ratio.

## PRINCIPLE OF TYPE ANALYSIS BY SULFURIC ACID

The principle underlying the use of sulfuric acid in type analysis (15) is the determination of a sufficient number of physical properties of a mixture before and after reaction with the acid to furnish calculation of the proximate composition, by appropriate combination of the data.

Complete structural type analysis of hydrocarbon mixtures involves complex ramifications (3) which are beyond the scope of absorption or extraction methods. For the purposes of routine analysis, however, any procedure which will give the per cent paraffins plus naphthenes, the paraffin-naphthene ratio, the per cent of olefins plus aromatics, and an independent determination of olefins, may be considered as yielding a type analysis.

Specifically, if accurate values of the per cent of saturates present and density and refractive index of the saturates can be obtained by the use of acid on a hydrocarbon mixture, then combined with molecular weight determination and bromine number determination on the original mixture, the composition can be expressed (16) in terms of per cent of paraffins, olefins, naphthenes and aromatics by established methods of interpreting specific refractivity (3, 5, 10) and bromine number (5, 15).

The primary object of the experimental work reported here has been to ascertain if the necessary data on volume per cent, density, and refractive index of the saturates present can be obtained by sulfuric acid extraction with sufficient accuracy, using acid strong enough to react completely with the olefins and aromatics. A secondary object has been to observe the effect of acid extraction on the aniline point, as a knowledge of this property is frequently of value (8).

Table I. Properties of Saturated Hydrocarbons Solvent 160-S

Gravity, ° A.P.I.	47.5	Density, $d_4^{20}$	0.786
Initial boiling point, ° F.	385	Dispersion ( $N_F - N_C$ ) × 10 <sup>4</sup> , 20° C.	77.4
5%	393	Specific dispersion	98.4
10%	396	Aniline point, ° F.	168.3
50%	407	Carbon, %	85.2
90%	425	Hydrogen, %	14.8
95%	433	Molecular weight	190
D.P.	437	Bromine No.	0.0
Flash point, ° F., T.C.C.	160	Napthenic rings per molecule, calculated	0.54
Refractive index, $d_4^{20}$	1.4368		

## MATERIALS AND APPARATUS

After a number of preliminary tests on known hydrocarbon mixtures, 101% sulfuric acid—i.e., anhydrous sulfuric acid containing 4.4 weight % of free sulfur trioxide—was selected as extracting agent.

A single saturated hydrocarbon fraction, a light kerosene fraction described in Table I, was employed in all the tests. Synthetic mixtures (refer to Table II) were made up using this fraction with various olefins and aromatics. The xylene used was Baker's c.p. grade, diisobutylene and cyclohexane were Eastman purified materials, and the aromatic amyl derivatives were samples obtained through the courtesy of Sharples Chemical Inc. All these unsaturated substances were used in their state of purity as received.

The extractions were carried out in graduated bottles similar to Stoddard solvent bottles (1), but specially made to contain 10% additional volume.

The optical measurements were made with an Abbe refractometer.



Table II. Extraction of Solutions of Unsaturated Hydrocarbons in Solvent 160-S by 101% Sulfuric Acid

Table III. Extraction Results of Simulated Hydrocarbons in Contact 100% of 10% Sulfuric Acid												
Samp. No.	Unsaturated Component	Vol. % Unsaturated Component	Ratio by Volume Acid:Oil	Absorption, Vol. %	Raffinate Properties			Original Solution Properties			Solute Properties	
					Refractive index, $20^{\circ}$ D	Density, $20^{\circ}$ 4	Aniline point, $^{\circ}$ F.	Refractive index, $20^{\circ}$ D	Density, $20^{\circ}$ 4	Aniline point, $^{\circ}$ F.	Refractive index, $20^{\circ}$ D	Density, $20^{\circ}$ 4
0	None	0.00	4:1	27.5	1.4330	0.7743	174.5	..	..	..	..	..
			3:1	20.5	1.4340	0.7772	173.0					
			2:1	13.5	1.4350	0.7803	171.5					
			Ext. 0:1	-0.5	1.4368	0.786	168.5					
1	Xylene	20.0	4:1	46.0	1.4328	0.7722	173.5	1.4478	0.8033	134.0	1.4953	0.8612
			3:1	39.5	1.4340	0.7760	172.0					
			2:1	33.0	1.4345	0.7800	170.5					
			Ext. 0:1	20.0	1.4368	0.7878	167.5					
2	Monoamyl benzene	20.0	4:1	34.0	1.4343	0.7780	172.0	1.4465	0.7999	139.5	1.4869	0.8577
			3:1	30.0	1.4350	0.7800	171.0					
			2:1	26.0	1.4358	0.7822	170.0					
			Ext. 0:1	18.0	1.4372	0.7864	167.8					
3	Diamyl benzene	20.0	4:1	32.0	1.4347	0.7789	172.0	1.4463	0.7996	150.5	1.4850	0.8537
			3:1	28.5	1.4351	0.7806	171.0					
			2:1	25.0	1.4358	0.7819	170.0					
			Ext. 0:1	18.0	1.4368	0.7849	167.8					
	Monoamyl naphthalene	20.0	4:1	36.0	1.4340	0.7769	173.5	1.4638	0.8220	135.5	1.5731	0.9641
			3:1	32.0	1.4347	0.7786	172.0					
			2:1	27.5	1.4354	0.7819	170.5					
			Ext. 0:1	19.2	1.4370	0.7879	167.5					
5	Diamyl naphthalene	20.0	4:1	37.5	1.4337	0.7758	173.5	1.4597	0.8162	142.5	1.5527	0.9340
			3:1	33.0	1.4346	0.7784	172.0					
			2:1	28.5	1.4354	0.7807	171.0					
			Ext. 0:1	19.5	1.4371	0.7858	168.6					
	Cyclohexene plus xylene	12.5	4:1	35.5	1.4350	0.7777	172.0	1.4447	0.7976	130.5	1.4470	0.8105
			3:1	32.0	1.4355	0.7806	170.0					
		12.5	2:1	28.5	1.4361	0.7830	168.5				1.4953	0.8612
			Ext. 0:1	21.5	1.4374	0.791	164.4					
	Diisobutylene	20	4:1	34.5	1.4327	0.7719	174.0	1.4330	0.7727	157.0	1.4106	0.7157
			3:1	30.0	1.4331	0.7750	172.5					
			2:1	25.5	1.4338	0.7780	171.0					
			Ext. 0:1	16.5	1.4350	0.7840	168.0					

For density determinations were made in 2- or 3-ml. pycnometer bottles, and aniline points were determined (2) using microburets and 5-ml. samples.

#### PROCEDURE

The extractions were carried out following the method of Thomas, Bloch, and Hoekstra (15), at ice-water temperature. Three extractions were made in each case, employing acid-oil ratios of 4 to 1, 3 to 1, and 2 to 1.

After the volume of hydrocarbon absorbed was measured, the extracts were discarded and the raffinate washed with sodium carbonate solution and dried over sodium carbonate. The density, refractive index, and aniline point of the washed and dried raffinate were determined and extrapolated graphically to a ratio of 0 to 1 acid-oil ratio.

The data obtained in the extraction experiments are recorded in Table II, including the extrapolated values.

#### ACCURACY OF SATURATES DETERMINATIONS

From the limited amount of data presented, it can be estimated that in the case of paraffin-naphthalene-benzene or paraffin-naphthalene-naphthalene mixtures, where the concentration of aromatic is 20% or less, the accuracy of the extrapolation method is approximately as follows:

Property	Estimated Accuracy
Volume per cent of saturates	$\pm 0.2\%$
Density of saturates	$\pm 0.005$
Refractive index of saturates	$\pm 0.0005$
Aniline point of saturates	$\pm 0.8^{\circ}$ F.

When the mixture contains olefinic derivatives at 12 to 20% concentration (see Table II), the inaccuracy is approximately doubled.

#### CALCULATION OF UNSATURATE PROPERTIES

Density and refractive index are approximately volume-additive properties in higher boiling hydrocarbon mixtures (4, 11, 14); hence the approximation equations below may be set up:

$$Fu^{(v)} = \frac{dm - ds}{du - ds} = \frac{Nm - Ns}{Nu - Ns}$$

Table III. Calculation of Properties of Unsaturated Compounds Mixed with Naphthenes and Paraffins, by Extrapolation Method

Unsaturated Compound	Data, Table II, Blend No.	Properties of Unsaturated Compound			
		Refractive Index, $20^{\circ}$ D	Density, $20^{\circ}$ 4	Calculated	Observed
Xylene	1	1.4918	1.4953	0.8653	0.8612
Monoamyl benzene	2	1.4888	1.4869	0.8614	0.8577
Diamyl benzene	3	1.4894	1.4850	0.8666	0.8537
Monoamyl naphthalene	4	1.5737	1.5731	0.9619	0.9641
Diamyl naphthalene	5	1.5530	1.5527	0.9417	0.9340
Diisobutylene	6	1.4230	1.4106	0.7160	0.7157

where

$Fu^{(v)}$  = volume fraction of unsaturates as determined by extrapolation method

$dm$  = density of original mixture

$ds$  = density of saturates, as extrapolated

$du$  = density of unsaturates

$Nm$  = refractive index of original mixture

$Ns$  = refractive index of saturates, as extrapolated

$Nu$  = refractive index of unsaturates

In Table III, the densities and refractive indices of the unsaturates used in the extraction tests have been calculated by the above formulas.

The agreement of calculated and observed values averages about  $\pm 0.005$  for the densities and  $\pm 0.004$  in the case of the refractive indices. Calculation of unsaturate aniline points was unsatisfactory.

#### DISCUSSION

It is evident from the experimental results in Table II that the accuracy of the extrapolation method is seriously reduced by olefins at high concentration. Other data on olefins at high concentration (above 20%) indicate that large proportions of olefinic compounds can make extrapolation indefinite.

For hydrocarbon mixtures containing 20% or less unsaturates, however, it appears to be a safe guide to consider that the extrapolation method fails when a linear continuation of the data to zero acid-oil ratio is not immediately apparent.



Application of the extrapolation method to hydrocarbon mixtures containing much more than 20% of olefins or aromatics is not practicable in any case with the present technique, inasmuch as the raffinate quantities would be too small for ordinary manipulation.

The extrapolation method of analysis appears at first sight to have the fundamental drawback of giving as unsaturated any substance which has at least one unsaturated group per molecule, regardless of the saturated character of the rest of the molecule. This is not actually a disadvantage in many cases, however, by virtue of the fact that the density and refractive index of the extracted unsaturated material can be approximated sufficiently to give a general idea of the nature of the unsaturates, especially if this information is supplemented by determinations of bromine number and optical dispersion (5, 16, 17).

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## Determination of Large Amounts of Manganese Modified Persulfate Method

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IN A STUDY of the reaction of ammonium persulfate with manganous salts in acid solution, it was discovered that by the addition of disodium hydrogen phosphate, the manganese can be oxidized and the excess persulfate decomposed by boiling; finally by the use of osmic acid, as recommended by Gleu (4), the permanganate may be stoichiometrically titrated with sodium arsenite solution. These modifications adapt the persulfate method to the accurate determination of manganese in larger amounts than has been possible heretofore and extend the usefulness of the procedure to the determination of manganese in such materials as manganese ores and ferromanganese. In this paper are given brief descriptions of the experimental work and a method of procedure for manganese in high-grade manganese ore.

Two major modifications in the usual persulfate method were adopted to permit accurate determination of moderately large amounts of manganese: (1) addition of disodium hydrogen phosphate to retard the decomposition of the permanganate, and (2) establishment of heating limits to decompose the excess persulfate, yet not affect the permanganate. Permanganate ion tends to decompose upon heating in an acid solution, the ionic equation for the reaction being



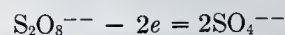
This equation shows that the reduction of the  $\text{MnO}_4^-$  involves the oxidation potential equation

$$E = E_0 - RT/3F \ln (\text{MnO}_4^-) (\text{H}^+)^4 \quad (2)$$

Assuming the oxidation potential of sodium to be positive, the  $E_0$  of this equation will be negative. Thus, upon examination, it is found that if the hydrogen-ion concentration, or the permanganate-ion concentration, is decreased, the oxidation po-

tential,  $E$ , becomes negatively smaller or actually larger, making the reduction less likely. Since it was desired to increase the limit on the amount of manganese present, the hydrogen-ion concentration or acidity was reduced through addition of disodium hydrogen phosphate.

While the reduction in hydrogen-ion concentration should, according to Equations 1 and 2, greatly reduce the rate of permanganate decomposition, it should not affect the rate of decomposition of the excess persulfate, since the ionic equation for the reduction of persulfate is



As can be observed from this equation, no hydrogen ions are involved in the process of reducing persulfate ion.

#### PROCEDURE

Although the maximum amount of manganese that can be present in a sample is still under investigation, it has been found that samples containing up to 0.05 gram of manganese can be effectively employed. Thus, an aliquot containing from 0.01 to 0.05 gram of manganese can be accurately analyzed by the procedure.

The sample first must be powdered, dried, and dissolved in aqua regia solution. This solution is filtered and the residue fused with c.p. sodium carbonate in a platinum crucible. If the carbonate turns green, manganese has been left in the precipitate and the fused mass must be dissolved in the filtrate. The resulting solution is added enough concentrated sulfuric acid to give 100 cc. of 6 *N* sulfuric acid solution when diluted to 250 cc.—approximately 33 grams or 18 cc. The aqua regia is evaporated off or the solution is evaporated to fumes of sulfur trioxide. The remaining solution is quantitatively washed into a 250-cc. volumetric flask and diluted to the mark. From this solution 25-cc. portions can be pipetted into flasks for analysis.

To each portion to be analyzed are added from 3 to 5 grams of disodium hydrogen phosphate, followed by approxima-



**Table I. Decomposition of Permanganate with and without Disodium Hydrogen Phosphate**

Experiment No.	Time of Heating Min.	Disodium Hydrogen Phosphate Added Grams	Sodium Arsenite Required Cc.	Sodium Arsenite Required for Blank Cc.	Variation of Trial from Blank Cc.
1	16 (water bath)	5	23.37	23.37	0.00 (none)
2	16 (water bath)	3	23.35	23.37	-0.02
3	16 (water bath)	1	22.89	23.37	-0.48
4	16 (water bath)	0	22.85	23.37	-0.52
5	5 (open flame)	0	22.77	23.37	-0.60
6	5 (open flame)	5	23.36	23.37	-0.01
7	15 (open flame)	5	23.36	23.37	-0.01

cc. of sirupy phosphoric acid. After the phosphate has completely dissolved, 10 cc. of freshly prepared 20% ammonium sulfate are added. The oxidation is catalyzed by 5 cc. of 0.1 N silver nitrate. The solution is now placed in a boiling water bath for 14 to 20 minutes or boiled gently over an open oxidizing flame 3.5 to 5 cm. in height for as close to 2 minutes as possible, then cooled immediately to 20° or 25° C. in a cold water bath. Then the solution is made strongly acid by adding 25 cc. of 6 N sulfuric acid. A few drops of the osmium tetroxide catalyst are added and the solution is ready for titration.

This may proceed in two different ways, by direct titration or by back-titration. The first method consists of running sodium arsenite titer into the solution being analyzed until it comes just colorless, or orange if the *o*-phenanthroline indicator is used. In the second method sodium arsenite is run into the solution until it is colorless, and a few milliliters are added in excess. This excess is then back-titrated with standardized potassium permanganate to the first sign of pink, or, if sodium phenylamine sulfate indicator is used, to a purple color. The amount of manganese present is calculated by a simple volumetric equation.

#### DISCUSSION

In performing the experimental work on this project, solutions of 0.1 N potassium permanganate, free from manganese dioxide, were made up, then reduced to manganous ions with hydrogen peroxide which was almost completely chloride-free. From this point the procedure described was followed.

Thus, the decomposition of the permanganate ion was determined by preparing the test solutions as above and heating for various times (Table I). Phosphate was absent or present in varying amount, and in no case did the procedure necessitate addition of any oxidation or reduction agents, since it was the permanganate decomposition that was being determined. The blanks, which were used to compare these results, were prepared by pipetting a definite amount of the potassium permanganate solution into a flask, acidifying, and titrating to an end point with the sodium arsenite.

The second modification in the procedure was determined solely through experiment. After the permanganate was kept from decomposing by the phosphate and the persulfate was again added, the comparative results began to run low once more. It is reasonable to assume that in some manner the decomposition of the persulfate had affected the oxidation of the manganese. Though what actually happens is not known, it was found that heating the test solution a so-called point of equilibrium could be established at which the persulfate is completely decomposed, but does not affect the permanganate formed by oxidation. This was accomplished by following the routine procedure up to the point which called for heating to decompose the persulfate. At this point, the solution was heated for various lengths of time in a water bath and over an open flame until the desired results were found. The results and comparison with the blank are given in Table II.

Many other trials were made to determine the limits on the amounts of reagents that might be used. In all cases the trials were identical with those reported in Table II, except that the reagent in question was varied from one extreme to the other. It was found that the amount of sirupy phosphoric acid used can vary between 7 and 13 cc. and still maintain the accuracy of the procedure. The amount of silver nitrate catalyst required may vary up to about 10 cc. but must not be less than 3 cc. The primary addition of sulfuric acid, which is often made while making up the original sample, should be between 0.08 and 0.025 equivalent. The minimum limit is established for this reagent, but the maximum limit may be somewhat larger, since no experimental trials were made above this point. The secondary addition of sulfuric acid is indefinite, as long as the test solution is made definitely acid.

As an indication of the accuracy of this procedure, several trials, using the method described, were made on manganese ore No. 25a, obtained from the Bureau of Standards (Table III). Another trial was made on the same sample using the bismuthate method (1, 5). The manganous ions were oxidized to permanganate by means of an excess of sodium bismuthate, the excess was filtered off, and the residue washed with dilute nitric acid. The test solution was titrated with sodium arsenite in a definitely acid solution.

#### REAGENTS

**OSMIUM TETROXIDE CATALYST.** Break a 1-gram capsule of osmium tetroxide beneath the surface of 390 cc. of 0.1 N sulfuric acid and make sure all has dissolved before filtering glass off. Osmium tetroxide emits poisonous vapors when standing in air. Preserve catalyst in a glass-stoppered bottle.

**SILVER NITRATE SOLUTION.** Dissolve approximately 17 grams of the silver nitrate salt in 1 liter of distilled water.

**AMMONIUM PERSULFATE SOLUTION.** Dissolve 20 grams of the persulfate in 100 cc. of distilled water at room temperature. This solution must be prepared fresh each time, since it decomposes upon standing.

**Table II. Effect of Time of Heating Solution on Decomposition of Ammonium Persulfate**

Experiment No.	Time of Heating Min.	Sodium Arsenite Required Cc.	Sodium Arsenite Required for Blank Cc.	Variation of Trial from Blank Cc.
1	12 (water bath)	23.49 (no constant end point)	23.37	-0.12
2	14 (water bath)	23.35	23.37	-0.02
3	16 (water bath)	23.37	23.37	0.00
4	18 (water bath)	23.36	23.37	-0.01
5	20 (water bath)	23.35	23.37	-0.02
6	22 (water bath)	23.31	23.37	-0.06
7	24 (water bath)	23.26	23.37	-0.11
8	5 (open flame)	23.28	23.37	-0.09
9	4 (open flame)	23.31	23.37	-0.06
10	3 (open flame)	23.34	23.37	-0.03
11	2 (open flame)	23.36	23.37	-0.01
12	1 (open flame)	23.51 (no constant end point)	23.37	-0.14

**Table III. Results of Actual Trials on Standard Samples**

Experiment No.	Type of Procedure	Manganese Determined %	Standard Value %	Error %
1	Persulfate	56.67	56.62	-0.09
2	Persulfate	56.65	56.62	-0.05
3	Persulfate	56.57	56.62	-0.09
4	Persulfate	56.62	56.62	None
5	Bismuthate	56.26	56.62	-6.38



**SODIUM ARSENITE SOLUTION.** Dissolve approximately 7 grams of sodium hydroxide pellets in as little cold water as possible, then dissolve exactly 4.9456 grams of pure dry arsenious oxide in this solution. Finally, neutralize with 1 *N* sulfuric acid, transfer to a 1-liter volumetric flask, and dilute to the mark. This gives an exactly 0.1 *N* solution of sodium arsenite.

**POTASSIUM PERMANGANATE SOLUTION.** Filter through a Jena glass crucible an approximately 0.1 *N* solution which has aged several days, and standardize against pure dry sodium oxalate.

#### ACKNOWLEDGMENT

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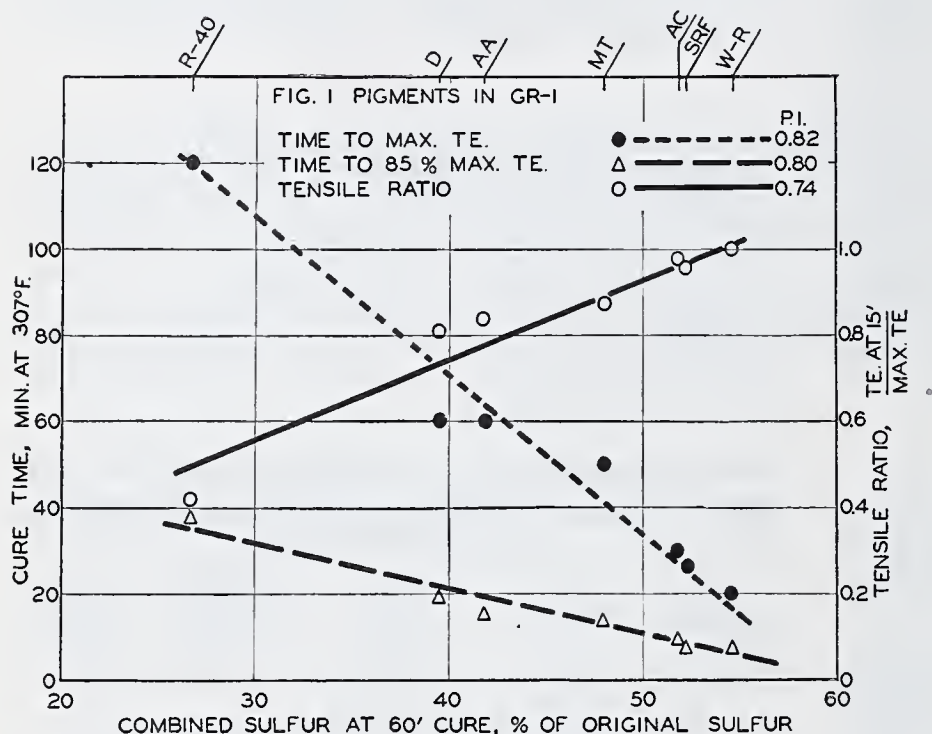
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## Determination of Rate of Cure of GR-I and Natural Rubber

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Indexes of rate of cure previously used in natural rubber and GR-S have been compared in GR-I (Butyl). Combined sulfur varies with cure time in a manner similar to that observed in natural rubber and GR-S. The T-50 temperature varies only slightly with cure time and does not appear to be satisfactory as an index of rate of cure in GR-I. Tensile ratio (tensile at an undercure/maximum tensile) appears to be a useful index of rate of cure in GR-I. For any series of stocks a convenient undercure is, as in the case of GR-S and natural rubber, a cure giving a tensile ratio about 0.60 for the stocks in the series having intermediate cure rates.

IN A previous publication two of the authors studied various methods for determining the rate of cure of natural rubber and GR-S (2). Evidence was presented to show that the tensile ratio, the ratio of tensile strength at an undercure to maximum tensile strength, was a useful index of rate of cure.

cure to maximum tensile strength, was a useful index of rate of cure.

#### TESTS IN NATURAL RUBBER ON PRODUCTION CARBON BLACKS

In order to examine further the applicability of the tensile ratio to practical rate of cure problems, tensile ratio, T-50 at 10 minutes, and T-50 at 45 minutes were determined for forty-four samples.

Table I. Tensile Ratio and T-50 for Forty-four Production Carbon Black Samples

(Expressed as per cent of a standard control)			
	Tensile Ratio, Tensile 10 Min./ Tensile 45 Min.	T-50, 10 Min.	T-50, 45 Min.
Average Grade AA (EPC)	112	117	111
Average Grade A (MPC)	106	103	111
Average Grade D (MPC)	103	99	111
Coefficient of correlation			
Tensile ratio vs. T-50 at 10-minute cure (all grades)			+0.98
Tensile ratio vs. T-50 at 45-minute cure (all grades)			-0.98
T-50 at 10-minute cure vs. T-50 at 45-minute cure (all grades)			-0.98

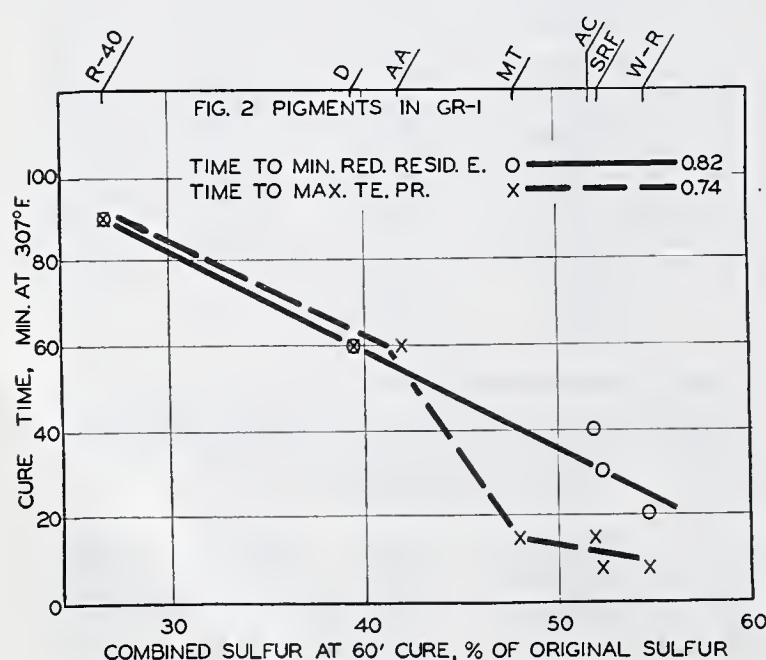




Table II. Pigments in GR-I

	Gum	Witecarb R	MT	Continex SRF	Continental AA	Continental D	Continental R-40	Acetylene Black
R-I (Butyl)	100	100	100	100	100	100	100	100
zinc oxide	5	5	5	5	5	5	5	5
stearic acid	1	1	1	1	1	1	1	1
thiuram	2	2	2	2	2	2	2	2
dimethylthiuram disulfide	1	1	1	1	1	1	1	1
mercaptobenzothiazole	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Witecarb R (W-R)	...	78	...	...	...	...	...	...
medium thermal (MT)	...	...	50	...	...	...	...	...
Continex SRF	...	...	...	50	...	...	...	...
Continental AA (EPC)	...	...	...	...	50	...	...	...
Continental D (MPC)	...	...	...	...	...	50	...	...
Continental R-40 (CC)	...	...	...	...	...	...	50	...
acetylene black (AC)	...	...	...	...	...	...	...	50
Cure at 307° F., Min.								
8	...	...	...	250	200	200	...	350
15	...	135	150	315	300	300	175	800
30	100	175	200	500	475	435	200	725
60	150	200	250	700	650	650	250	925
90	175	200	285	735	800	850	350	975
180	200	200	325	735	925	880	400	1000
Tensile at Break, Pounds per Square Inch								
8	280 <sup>a</sup>	1760	1075	1675	1200	1380	390	1250
15	1100 <sup>a</sup>	1875	1460	1820	1960	2160	740	1570
30	2530 <sup>a</sup>	1875	1600	1900	2150	2475	1400	1600
60	2530 <sup>a</sup>	1825	1675	1850	2335	2670	1675	1600
90	2000 <sup>a</sup>	1800	1525	1800	2300	2630	1750	1600
180	1975 <sup>a</sup>	1800	1525	1750	2300	2650	1700	1500
Elongation at Break, %								
8	1300 <sup>a</sup>	1000	960	935	1000	1110	1200	825
15	1200 <sup>a</sup>	875	900	815	915	960	1100	750
30	1050 <sup>a</sup>	835	815	725	850	925	950	650
60	875 <sup>a</sup>	815	775	675	800	885	925	600
90	825 <sup>a</sup>	800	735	650	715	790	900	590
180	825 <sup>a</sup>	800	675	635	685	770	800	575
Breaking Set, %								
8	...	30	25	30	60	68	150	50
15	...	25	25	25	50	50	75	38
30	...	25	25	25	43	45	58	35
60	...	23	20	25	35	35	55	35
90	...	20	15	20	27	35	50	30
180	...	18	15	20	25	30	50	19
Tear, Pounds per Square Inch								
8	40	200	90	200	185	280	85	200
15	45	150	85	200	325	405	135	215
30	30	145	70	190	395	475	175	250
60	30	125	50	150	415	495	200	220
90	35	115	50	160	385	440	200	220
180	25	125	75	160	390	435	...	...
Durometer, Instantaneous								
8	24	40	40	48	51	51	51	51
15	26	40	40	49	53	55	51	55
30	27	42	42	50	54	56	51	56
60	30	45	45	55	56	59	53	60
90	30	46	45	53	57	62	54	60
180	30	45	43	51	57	62	51	60
Durometer, 30-Second Reading								
8	16	30	30	39	38	39	35	41
15	21	34	35	41	43	46	39	47
30	22	38	39	45	45	46	40	51
60	28	40	41	50	50	52	40	57
90	29	41	41	50	51	55	42	57
180	29	41	40	47	51	56	42	56
Rebound at 100° C., %, Bashore								
8	37	30	34	28	21	19	18	22
15	38	31	36	29	22	20	18	23
30	43	32	37	30	23	21	20	23
60	46	32	39	31	24	23	21	25
90	48	33	41	33	26	25	22	25
180	46	32	40	31	26	26	23	24
Abrasion Loss, Du Pont, Ce. per Hp.-Hour								
30	<sup>b</sup>	1320	1590	346	...	...	...	229
60	<sup>b</sup>	1020	760	265	401	424	...	160
90	...	...	...	...	...	...	534	...
180	<sup>b</sup>	937	663	249	329	361	481	157
Heat Buildup, ° F. <sup>c</sup>								
90	<sup>d</sup>	195	<sup>d</sup>	201	...	...	...	253
150	...	...	...	...	257	268	<sup>e</sup>	...
Williams Plasticity, Inch								
..	0.110	0.118	0.116	0.124	0.141	0.150	0.166	0.139
Tubing Rate, Grams per Minute <sup>f</sup>								
..	15.0	26.0	24.0	27.0	22.2	20.8	19.2	29.7
T-50, ° C.								
15	-44.0	-39.8	-37.5	-29.3	-18.9	-17.5	-10.8	-14.0
90	...	-43.0	...	...	-25.5	-23.8	-14.3	...
Combined Sulfur, % of Original Sulfur								
60	49.8	54.6	47.9	52.3	41.8	39.5	26.6	51.9
Tensile Ratio, 15-Minute/Maximum								
..	...	1.0	0.87	0.96	0.84	0.81	0.42	0.98

ples of channel black collected weekly from several different operating units. The blacks were milled in the following test formulation which was cured at 280° F.:

Smoked sheet	100
Stearic acid	4.0
Pine tar	1.5
Sulfur	3.0
Phenyl-β-naphthylamine	1.0
Zinc oxide	3.0
Mercaptobenzothiazole	1.0
Carbon black	50.0

Under the conditions used all the channel blacks reached a tensile in 45 minutes sufficiently close to the maximum tensile so that the more readily determined ratio (tensile at 10 minutes/tensile at 45 minutes) could be used for tensile ratio in place of (tensile at 10 minutes/maximum tensile). All samples were tested alongside a standard control and the average values reported in Table I are expressed as per cent based on the control. Percentages are calculated so that higher values indicate faster cure rate.

Considering the average values of the three grades of black tested, the tensile ratio and T-50 at 10-minute cure indicate decreasing rate of cure in going from AA to D. This is in agreement with actual experience. T-50 at 45 minutes, however, does not follow the expected trend. Likewise, the coefficient of correlation between tensile ratio and T-50 at 10 minutes is +0.30, which for the number of stocks used shows some interdependence, there being less than one chance in twenty that this correlation could arise through random sampling errors. On the other hand, T-50 at 45 minutes shows no correlation with either tensile ratio or T-50 at 10 minutes. Just why the T-50 at 10 minutes and T-50 at 45 minutes should agree so poorly for these stocks is not clear; however, the evidence indicates that both tensile ratio and T-50 at 10 minutes

<sup>a</sup> Results questionable owing to tendency of gum stock to tear in tensile jaws.

<sup>b</sup> Test block crumbled when abraded.

<sup>c</sup> Heat rise of center of test plug over room temperature (70° ± 3° F.) on St. Joe flexometer after 20 minutes at 475 pounds' vertical load and 0.130-inch horizontal deflection. Face plate temperature of 100° ± 2° F. used at beginning of each test.

<sup>d</sup> Test plugs blew out in less than 5 minutes under conditions of test.

<sup>e</sup> Test plug could not be successfully cured.

<sup>f</sup> 0.5-inch Royle tuber, 3/16-inch die, 24 r.p.m. screw speed, and 170° F. barrel temperature.



can be used as indexes of cure rate. The tensile ratio has the advantage of being very simply obtained from tensile data which are usually determined anyhow in evaluating a stock.

#### RATE OF CURE OF GR-I

The rate of cure of GR-I (Butyl) was studied, following the approach previously used for natural rubber and GR-S (2). Two series of stocks were used in this work. The first series, formula and tests on which appear in Table II, consists of one inorganic and various carbon pigments and covers a wide range of cure rates. The second series (Table III) includes only channel carbon blacks and covers a narrower cure range. A loading of 25.6 volumes per 100 volumes of GR-I was used for all pigments. Physical properties, T-50 tests, and combined sulfur data are included in the tables. A.S.T.M. standard test conditions were used except where otherwise specified. A different shipment of GR-I was used in each set. Although both sets are consistent within themselves, appreciable differences were found between the two sets. These differences may be due to variation in the polymer or to unintentional differences in processing the two sets.

Combined sulfur was calculated from the sulfur originally present by subtracting the free sulfur determined by the sodium sulfite extraction method (1, 4).

The sulfur determinations are subject to two principal errors: (1) the tetramethylthiuram disulfide in the formula may interfere, giving high results for free sulfur, and (2) slow diffusion through GR-I may lead to incomplete extraction of the free sulfur in the standard 2-hour extraction time. Determinations were therefore carried out at various extraction times with the results shown in Table IV. In the case of the uncured stock, a value equal to essentially all the free sulfur is obtained in 8 hours. Since for the cured stock the per cent of sulfur extracted also appears to be leveling off at about this time, the 8-hour extraction was adopted throughout. (Since this work was completed a new method for determination of sulfur in Butyl based on methyl ethyl ketone extraction has appeared, 5.)

The physical test data in Tables II and III were used to determine time to reach maximum tensile, tensile product, tear, and rebound; time to reach minimum reduced residual elongation (breaking set divided by tensile); time to reach 85% and other percentages of maximum physical properties; tensile ratio (ten-

Table III. Channel Blacks in GR-I<sup>a</sup>

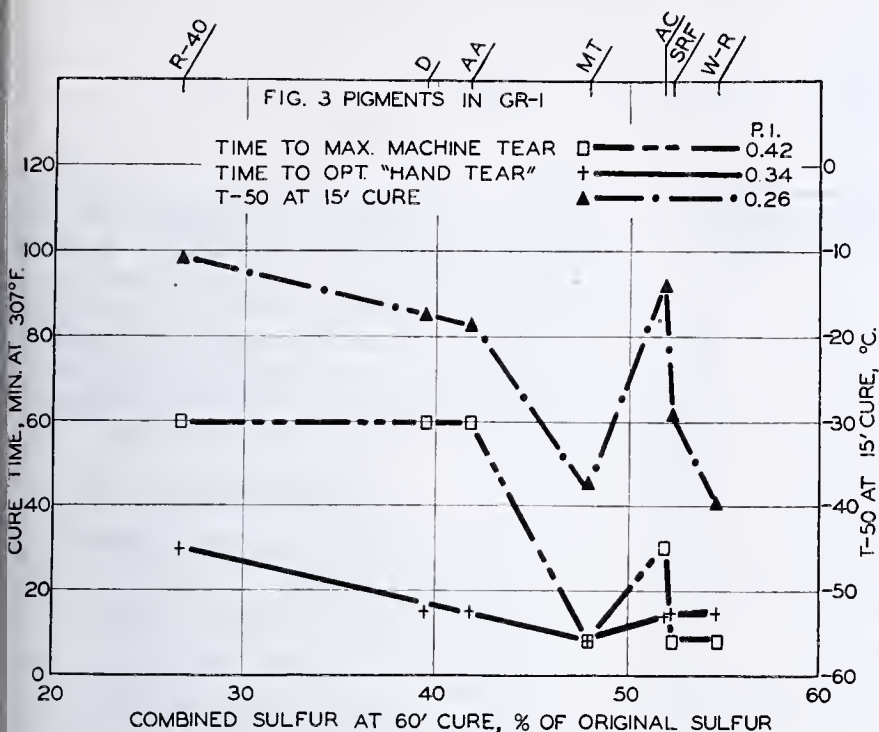
	Continental AAA	Continental AA	Continental A	Continental D	Continental F	Continental R-20	Continental R-30	Continental R-40
GR-I (Butyl)	100	100	100	100	100	100	100	100
Zinc oxide	5	5	5	5	5	5	5	5
Stearic acid	1	1	1	1	1	1	1	1
Sulfur	2	2	2	2	2	2	2	2
Tetramethylthiuram disulfide	1	1	1	1	1	1	1	1
Mercaptobenzothiazole	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Continental AAA (EPC)	50	...	...	...	...	...	...	...
Continental A (MPC)	...	50	...	...	...	...	...	...
Continental D (MPC)	...	...	50	...	...	...	...	...
Continental F (HPC)	...	...	...	50	...	...	...	...
Continental R-20 (CC)	...	...	...	...	50	...	...	...
Continental R-30 (CC)	...	...	...	...	...	50	...	...
Continental R-40 (CC)	...	...	...	...	...	...	50	...
Cure at 307° F., Min.								
8	250	180	200	...	...	210	150	75
15	375	280	250	320	475	300	300	125
30	650	400	400	450	550	450	375	250
60	750	600	600	625	600	650	550	350
90	800	700	750	775	675	700	725	400
180	900	800	900	950	800	875	775	525
Modulus at 400% Elongation, Pounds per Square Inch								
8	1275	775	925	800	800	1075	525	335
15	1750	1400	1500	1600	1600	1725	1450	550
30	2010	1675	2100	2250	2225	2475	2300	1100
60	2325	2000	2400	2425	2500	2650	2525	1350
90	2300	1950	2400	2475	2475	2725	2600	1400
180	2250	1925	2325	2400	2400	2725	2575	1535
Tensile at Break, Pounds per Square Inch								
8	1120	1055	1200	1170	930	950	900	800
15	980	890	1025	1035	835	860	880	730
30	880	825	895	870	800	850	950	775
60	795	760	800	815	790	775	845	770
90	700	700	740	740	755	750	805	700
180	700	655	705	705	740	740	780	700
Elongation at Break, %								
8	53	53	68	75	75	40	110	165
15	40	38	53	50	53	50	92	75
30	32	35	45	45	50	50	70	70
60	30	30	38	38	50	45	70	60
90	25	33	35	33	38	38	65	60
180	25	25	30	30	35	37	58	60
Breaking Set, %								
8	260	150	145	130	145	250	120	135
15	380	225	235	350	310	395	175	145
30	425	315	480	475	430	495	430	190
60	475	365	495	485	475	495	545	195
90	450	350	460	475	485	500	545	200
180	450	350	460	415	475	500	530	215
Tear, Pounds per Inch								
8	47	46	45	50	40	47	45	25
15	49	48	47	51	45	50	50	30
30	50	49	49	51	49	52	51	35
60	52	50	52	55	52	54	53	50
90	52	51	54	56	51	55	57	49
180	52	50	55	54	50	54	57	50
Durometer, Instantaneous								
8	35	35	31	36	28	38	33	20
15	40	40	39	41	39	43	40	22
30	43	41	41	45	40	45	45	26
60	46	45	46	48	47	48	46	40
90	48	48	50	50	46	50	50	41
180	48	45	50	50	48	49	50	43
Durometer, 30-Second Reading								
8	24	25	23	23	25	22	19	19
15	25	27	24	25	25	23	21	21
30	31	33	30	29	27	26	23	26
60	32	34	30	30	28	25	22	24
90	33	33	31	30	29	27	25	25
180	31	32	29	28	28	27	24	24
Rebound at 100° C., %, Bashore								
150	242	242	247	251	248	256	b	c
Tubing Rate, Grams per Minute								
..	18.4	18.8	18.2	18.6	17.3	14.6	14.7	16.4
T-50, ° C.								
15	-21.3	-21.0	-20.4	-20.5	-20.2	-19.6	-16.0	-10.8
90	...	...	-25.0	-24.4	-24.0	-25.5	...	...
Combined Sulfur, % of Original Sulfur								
30	38.0	36.2	31.9	30.7	...	...	30.2	...
60	45.4	43.6	40.0	40.0	40.0	40.2	41.6	32.6
Tensile Ratio (15-Minute/Maximum)								
..	0.75	0.70	0.63	0.65	0.64	0.63	0.56	0.36

<sup>a</sup> Same test conditions as listed in Table II.

<sup>b</sup> Blew out in 18.5 minutes under conditions of test.

<sup>c</sup> Test plug would not cure properly even after 4 hours at 307° F.





ile at 15 minutes/maximum tensile); and tensile product ratio (tensile product at 15 minutes/maximum tensile product). The 5-minute cure was selected, as this cure gave tensile ratios between 0.30 and 1.00 for all stocks and ratios of about 0.60 for stocks having intermediate cure rates. Time to reach optimum cure as judged by hand tear was also determined. No attempt was made to determine the break in the modulus versus cure time curve, as the change in slope of this curve is very gradual for the GR-I formulation used. The indexes of rate of cure which have been proposed in the literature and also those indexes which were found to correlate closely with combined sulfur are plotted against combined sulfur in Figures 1 to 5. Prediction indexes (P.I. =  $-\sqrt{1-r^2}$ , where  $r$  is the coefficient of correlation) showing the extent of correlation of the various indexes of rate of cure with combined sulfur are tabulated on the figures.

In both the pigment and channel black series, tensile ratio shows a high degree of correlation with combined sulfur. Time to 85% maximum tensile, time to optimum tensile, time to optimum machine tear, and time to optimum tensile product also show a significant correlation within the 95% confidence limits for both sets. The minimum in the reduced residual elongation versus cure-time curve is not an absolute minimum but a mathematical minimum followed by a maximum and then a further drop to lower levels. For this reason, the minimum is difficult to determine unless a considerable number of cures are available in the minimum region. The minimum could be determined for only five of the pigment series and two of the channel black series.

The GR-I gum stock was not included in the graphs or calculations because of the questionable character of most of the stress-strain data. This stock tore so easily that it was extremely difficult to prevent test dumbbells from tearing in the jaws of the tensile machine. The values reported were obtained by using a narrow die (0.090 instead of 0.250 inch in width) and disregarding all samples which did not break near the center of the test dumbbell. Judging from the combined sulfur results, the T-50 value for the gum stock also

appears too low compared with the T-50 values for the loaded stocks. In natural rubber, too, it has been found that the T-50 cannot be used in comparing the rate of cure of a gum stock with that of loaded stocks (2). In such a comparison, T-50 is out of line with practically all other indexes of rate of cure.

Of those indexes showing good correlation with combined sulfur, tensile ratio is for many purposes one of the most convenient to determine. It therefore appears that tensile ratio is a useful index of rate of cure in GR-I as well as in natural rubber and GR-S.

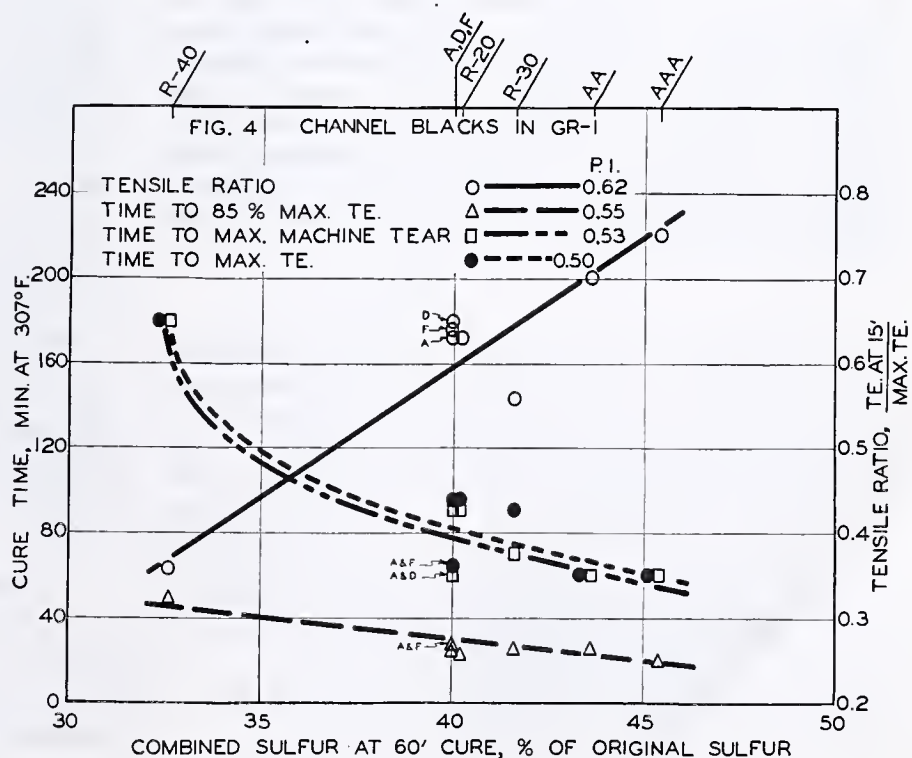
The T-50 temperature decreases slightly with increasing cure time. The decrease is greater than for GR-S but much less than for natural rubber. In view of the small difference between T-50 at 15 minutes and T-50 at 90 minutes, the rather large differences between stocks containing various pigments were surprising. Apparently this behavior may be due to the effect of some other property of the stock, such as set, on T-50, rather than to rate of cure. In any case it appears doubtful that the T-50 temperature can be used as a satisfactory index of rate of cure for GR-I.

The T-50 temperature may be associated with the melting point of crystallites formed at low temperature in the stretched polymer. Since GR-S does not crystallize and GR-I crystallizes to only a limited extent at high elongation, we might expect them

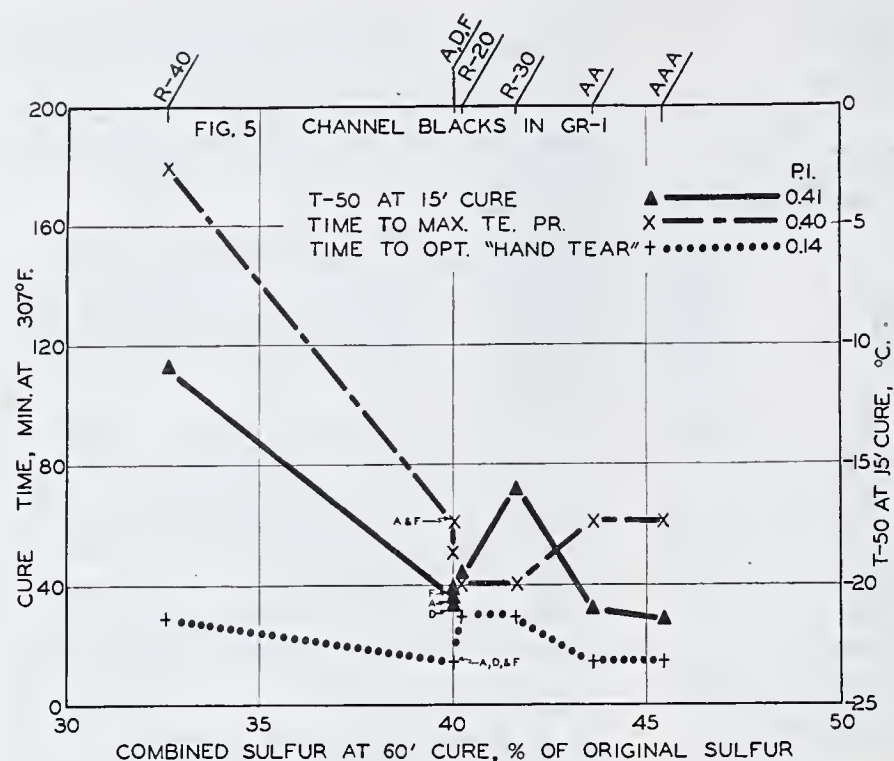
Table IV. Effect of Extraction Time on Amount of Sulfur Extracted  
(Expressed as per cent of original sulfur)

Stock,	Extraction Time				
	2 Hours	4 Hours	5.5 Hours	8 Hours	16 Hours
Continental AA in GR-I (uncured)	75.3	...	95.2	99.4	...
Continental D in GR-I <sup>a</sup> (60-min. cure)	55.5	57.9	...	57.9	58.9

<sup>a</sup> A different cure than reported in Table II.







to show, respectively, no and very little variation in T-50 temperature with time of cure. In this connection it might be of

interest to determine the temperature at which the x-ray crystal pattern disappears in stretched natural rubber as a function of time of cure. (The per cent crystalline phase at a given elongation has been found to increase sharply at very early cures followed by a gradual decrease, 3. However, this behavior is not necessarily connected with the temperature at which crystallites melt.)

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## Quantitative Determination of Extractable Gossypol in Cottonseed and Cottonseed Meal

### A Spectrophotometric Method

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The reaction of gossypol with antimony trichloride in ether and chloroform extracts of cottonseed is specific and the reaction product is sufficiently stable to permit accurate spectrophotometric determination of the gossypol concentration of such extracts. A rapid direct method for determination of free gossypol in cottonseed is reported, in which the gossypol is extracted by equilibrating ground cottonseed and chloroform, treating the extract with concentrated hydrochloric acid, and applying the antimony trichloride reaction directly to the treated extract.

IT WAS recently shown (1) that gossypol reacts with antimony trichloride in chloroform to form a soluble red product having an absorption curve which exhibits a broad maximum at 510 to 520  $m\mu$ . It was shown further that the magnitude of the extinction at the maximum is directly proportional to the concentration of gossypol in the test solution and that therefore the absorption spectrum of the antimony trichloride reaction product could be used as a quantitative measure of gossypol in solution. However, it was found that the absorption curves of the antimony trichloride reaction products with cottonseed extracts differed somewhat from those of similar products obtained with solutions of pure gossypol.

Subsequent investigation of the antimony trichloride reaction

products with cottonseed extracts has shown that in most cases the reaction product exhibits, initially, an absorption curve identical with that obtained with pure gossypol. Antimony trichloride evidently reacts more rapidly with gossypol than with the interfering reactants which may be present in cottonseed extracts. Consequently, when the absorption maximum at 510 to 520  $m\mu$  of the reaction product of antimony trichloride with cottonseed extract is determined before interfering reactions have developed, the height of this maximum serves as a quantitative measure of the concentration of gossypol in such an extract.

In some hydraulic press-cake meals, as well as in some raw cottonseed, much of the "free" gossypol occurs in the form of an orange-colored pigment (2) which can be converted into gossypol by treating the extracts with concentrated hydrochloric acid. Following such treatment, these extracts react with antimony trichloride in a manner completely analogous to that of cottonseed extracts containing negligible amounts of the orange-colored pigment.

#### ANTIMONY TRICHLORIDE REACTION WITH PURE GOSSYPOL

The gossypol-antimony trichloride reaction is carried out as follows:

To 1 ml. of a chloroform solution of purified gossypol in a glass-stoppered absorption cell there are added 1 drop of acetic anhydride

<sup>1</sup> On military leave for duty with U.S.N.W.R.



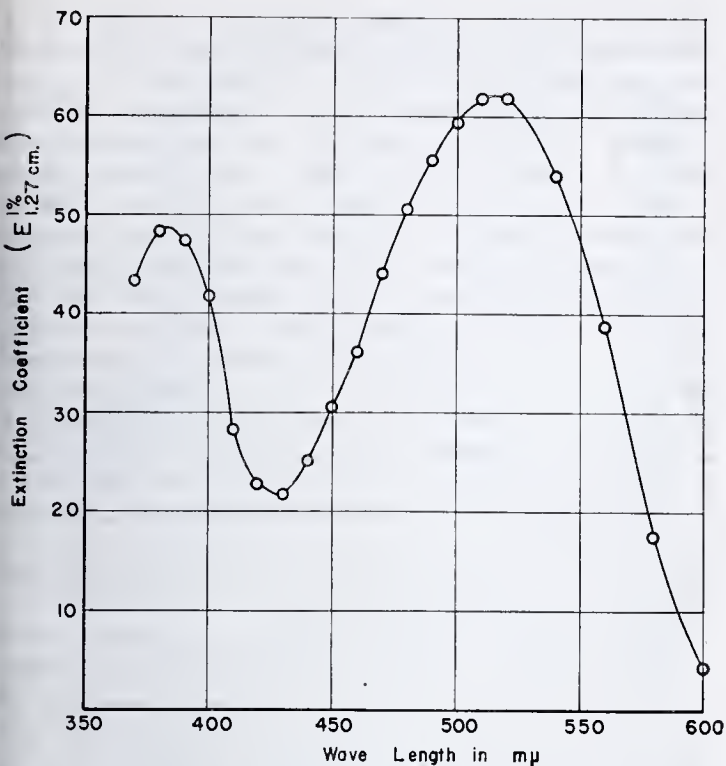


Figure 1. Absorption Curve of Antimony Trichloride Reaction Product with Pure Gossypol

dride and 5 ml. of a saturated chloroform solution of antimony trichloride. The acetic anhydride is added to prevent the development of the hazes produced by moisture in the extracts. For the same reason it is desirable to mix the reagents directly in the absorption cells and thus avoid unnecessary exposure to atmospheric moisture. The transmission of the solution is measured against that of a blank which consists of 1 ml. of chloroform, 1 drop of acetic anhydride, and 5 ml. of a saturated chloroform solution of antimony trichloride.

The volumes of reagents indicated were chosen to suit the capacity of the cells designed for the Coleman double monochromator spectrophotometer which was used for all absorption measurements reported here. The Coleman spectrophotometer is equipped with cells having an estimated optical depth of 1.27 cm. and all extinction coefficients were, therefore, expressed in terms of this optical depth rather than the customary 1.0-cm. depth. Extinction coefficients are defined by the equation  $E = \frac{\log I_0/I}{cl}$  where  $\log I_0/I$  is the extinction,  $I_0$ , the intensity of light transmitted by the blank,  $I$  the intensity of light transmitted by the solution,  $c$  the concentration of the solution, and  $l$  the length of the path of light through the liquid. The extinction coefficients were calculated in terms of the optical depth of the absorption cells used (1.27 cm.) and, for convenience in calculating the concentration of gossypol in test extracts, they were expressed in terms of the concentration in grams of gossypol per 100 ml. of the original solution to which the acetic anhydride and the antimony trichloride solution were added.

The absorption curve of the antimony trichloride reaction product with pure gossypol is shown in Figure 1. The curve is characterized by two maxima, a broad one in the visible range at 510 to 520  $m\mu$ , and a sharper one in the near ultraviolet at 380  $m\mu$ , and by a minimum at 430  $m\mu$ .

As shown in Figure 2, a straight-line relationship exists between the values of  $\log_{10} I_0/I$  and the concentration of gossypol at 520, 430, and 380  $m\mu$  for concentration of gossypol in the original solution ranging from 0.004 to 0.016 gram per 100 ml. of solution. These values were obtained with solutions of three preparations of gossypol prepared according to two independent procedures (1, 2).

The existence of two well-defined, widely separated absorption maxima permitted the mathematical characterization of the absorption spectrum (4, 15) of the gossypol-antimony trichloride reaction product in terms of the ratios of the magnitudes of the maxima to each other and to the minimum. These ratios can

be used for establishing the specificity (4, 15) of the reaction for gossypol contained in extracts of cottonseed. As shown in Table I, the value of the ratio  $R_a$ ,  $\log_{10} I_0/I$  at 520  $m\mu$  to  $\log_{10} I_0/I$  at 430  $m\mu$ , is  $2.68 \pm 0.23$ , and the ratio  $R_b$ ,  $\log_{10} I_0/I$  at 520  $m\mu$  to  $\log_{10} I_0/I$  at 380  $m\mu$ , is  $1.22 \pm 0.07$  for pure gossypol in the antimony trichloride reaction.

The absorption spectrum in the range 370 to 600  $m\mu$  reaches its maximum development within 10 minutes after the reagents are mixed and is stable for at least 24 hours.

In contrast to the reaction of gossypol, the orange-colored pigment from cottonseed (2) does not form a stable reaction product with antimony trichloride. When a chloroform solution of the orange-colored pigment is first treated with concentrated hydrochloric acid and then reacted with antimony trichloride, the reaction product exhibits an absorption spectrum identical with that of the gossypol-antimony trichloride reaction product with respect to both positions and ratios of maxima and minimum.

Because of its broadness and its position in the visible wavelength range, the absorption maximum at 510 to 520  $m\mu$  serves best as a standard for computing gossypol concentrations in test solutions. When dilutions ranging from 0.004 to 0.016% gossypol of nine different solutions of three preparations of pure gossypol obtained by two different procedures (1, 2) were used, the values for  $E_{1.27}^{1\%}$  at 520  $m\mu$  shown in Table I were obtained. From these data it may be concluded that the value of  $E_{1.27}^{1\%}$  at 520  $m\mu$  is  $65.5 \pm 1.9$  for gossypol in the antimony trichloride test where the concentration of gossypol is expressed in terms of grams per 100 ml. of the original solution.

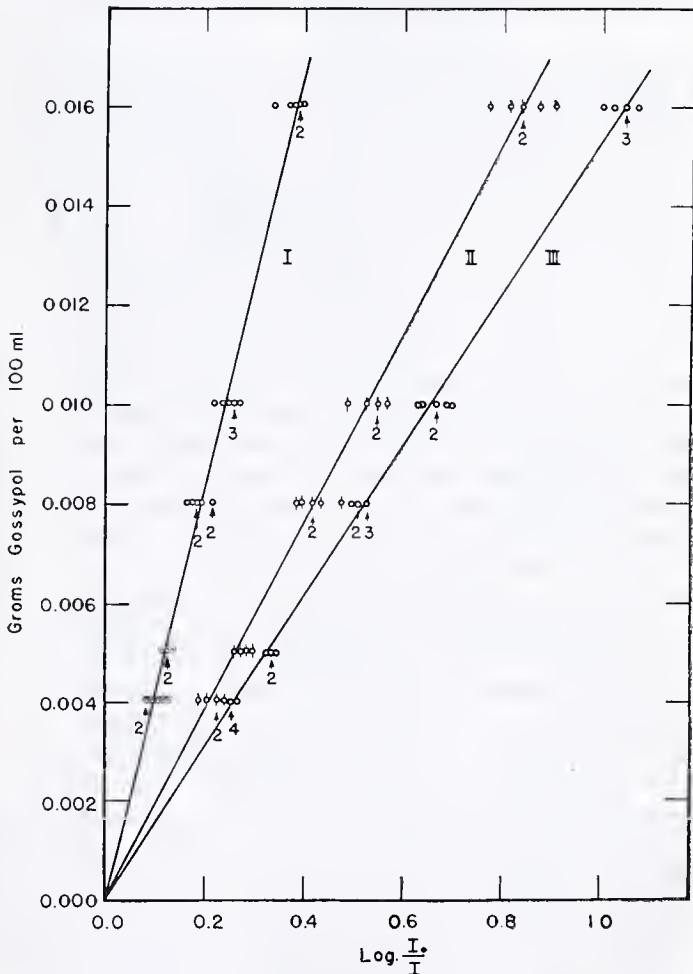


Figure 2. Demonstration of Beer's Law in Gossypol-Antimony Trichloride Reaction

- I. Extinction at 430  $m\mu$  vs. gossypol concentration
  - II. Extinction at 380  $m\mu$  vs. gossypol concentration
  - III. Extinction at 520  $m\mu$  vs. gossypol concentration
- Arabic numerals indicate multiplicity of points obtained in independent tests.



Table I. Absorption Spectrum of Antimony Trichloride Reaction Product with Pure Gossypol

Gossypol Preparation	Concentration, Gram/100 Ml.	$R_a^a$	$R_b^b$	$E_{1.27\text{ cm. at } 520\text{ m}\mu}$
FSBP	0.010	2.49	1.17	63.8
	0.010	2.88	1.29	62.9
	0.016	2.72	1.15	62.5
	0.008	3.16	1.29	63.7
	0.008	2.84	1.28	61.9
	0.004	3.02	1.37	65.0
Y <sub>00</sub>	0.010	2.69	1.22	69.9
	0.005	2.49	1.20	68.4
	0.010	2.69	1.26	65.8
	0.005	2.57	1.20	67.4
	0.005	2.79	1.24	64.6
	0.016	3.03	1.32	63.9
	0.008	2.82	1.24	64.5
	0.004	2.49	1.15	65.0
FSB <sup>c</sup>	0.016	2.80	1.27	66.9
	0.016	2.77	1.16	65.4
	0.016	2.66	1.29	65.4
	0.008	2.58	1.21	63.6
	0.008	2.53	1.15	66.3
	0.008	2.47	1.28	66.3
	0.004	2.11	1.11	63.0
	0.004	1.99	1.08	65.0
	0.004	2.68	1.34	65.0
	0.010	2.69	1.26	68.8
	0.010	2.61	1.19	66.8
	0.010	2.86	1.19	66.8
	0.005	2.56	1.10	65.6
	0.005	2.69	1.22	67.4
Y <sub>00</sub> <sup>c</sup>	0.016	2.73	1.24	65.4
	0.008	2.83	1.22	66.3
	0.004	2.96	1.27	66.0
Average values		2.68	1.22	65.5
Deviation from mean		$\pm 0.23$	$\pm 0.07$	$\pm 1.9$

<sup>a</sup>  $R_a = \log_{10} I_0/I$  at 520 m $\mu$  to  $\log_{10} I_0/I$  at 430 m $\mu$ .<sup>b</sup>  $R_b = \log_{10} I_0/I$  at 520 m $\mu$  to  $\log_{10} I_0/I$  at 380 m $\mu$ .<sup>c</sup> Solutions treated with concentrated hydrochloric acid before reaction with antimony trichloride.

cottonseed, produce an orange or yellow color instead of the characteristic red reaction product with antimony trichloride. The absorption spectra of these reaction products are very unstable but show certain definite tendencies as illustrated in curves 1 and 2 of Figure 3. The first maximum shifts from 510 to 520 m $\mu$  to 490 m $\mu$ ; a new maximum appears at 450 m $\mu$ ; and the maximum at 380 m $\mu$  shifts to 390 m $\mu$ . Such curves appear to represent the result of superposing on the gossypol-antimony trichloride absorption curve, which has maxima at 380 m $\mu$  and 510 to 520 m $\mu$ , the absorption curve of the unstable reaction product of antimony trichloride with the orange-colored pigment (2), which has a maximum at 450 to 460 m $\mu$ , and that of the unstable antimony trichloride reaction product with at least one other pigment, which has maxima at 390 and 490 m $\mu$ . In confirmation of this explanation of the shape of these curves, it was observed that when such extracts were treated with concentrated hydrochloric acid prior to their reaction with antimony trichloride, reaction products which showed typical gossypol-antimony trichloride absorption curves were obtained, as shown in Table II.

Thus it has been possible to apply absolute criteria for the establishment of the specificity (4, 15) of the antimony trichloride reaction for gossypol in cottonseed extracts. It is evident that any concurrent reactions of antimony trichloride to form colored products with extractable components of cottonseed other than gossypol would alter the shape of the curve and thus change the values of the ratios. In the absence of such interfering reactions the absorption serves as a direct measure of the gossypol concentration in the extracts.

#### ANTIMONY TRICHLORIDE REACTION APPLIED TO COTTONSEED EXTRACTS

The complete absorption curves, in the region of 370 to 600 m $\mu$ , of freshly prepared mixtures of chloroform extracts, and of chloroform solutions of ether extracts, of many cottonseeds with antimony trichloride are almost identical with the absorption curve obtained with pure gossypol and antimony trichloride. As the reaction mixtures stand, absorption increases in the shorter wave-length region and the whole character of the curves changes. In order to establish the specificity of the reaction it was necessary to read the spectra as soon as possible after the reagents were mixed. Therefore, only the absorptions at the critical wave lengths, 530, 520, 510, 500, 430, 390, 380, and 370 m $\mu$ , were determined and these were read as rapidly as possible after the expiration of the 10-minute induction period. With this procedure it was found, as shown in Table II, that the absorption spectra of fresh mixtures of antimony trichloride with chloroform solutions of both chloroform and ether extracts of cottonseed are analogous to the gossypol-antimony trichloride absorption spectrum. The maxima and the minimum occur at the same wave lengths and are of the same relative magnitudes, as shown in Table II. The spectra listed in the table were chosen to illustrate both the best and worst agreement of the  $R_a$  and  $R_b$  values of the extract reaction products with those of the pure gossypol reaction product. Several examples of values obtained from the reaction performed with duplicate samples of the same extract and with duplicate extracts of the same cottonseed are included. The duplication of the values of  $E$  at 520 m $\mu$ , even though the values of the ratios are not duplicates, demonstrates that the value of  $E$  at 520 m $\mu$  is an accurate measure of the gossypol concentration.

Chloroform and ether extracts of some hydraulic-pressed cottonseed meals, as well as of some raw

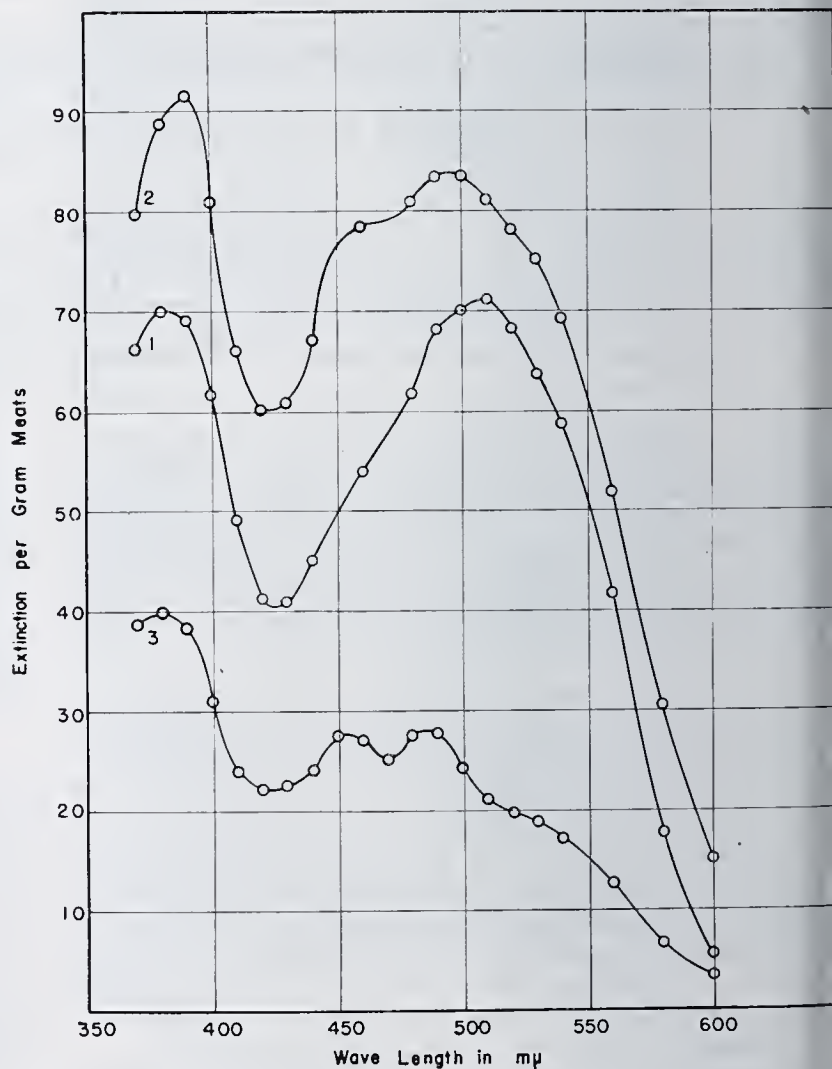


Figure 3. Absorption Curves of Antimony Trichloride Reaction Product with Cottonseed Extracts

1. Reaction product with chloroform extract 15 to 20 minutes after mixing reactants
2. Reaction product with chloroform extract 24 hours after mixing reactants
3. Reaction product with Skellysolve F extract 24 hours after mixing reactants



**Table II. Absorption Spectrum of Antimony Trichloride Reaction Product with Gossypol in Cottonseed Extracts**

Cottonseed Sample	Extraction Solvent	$R_a$	$R_b$	$E$ at 520 $m\mu$ per Gram	Per Cent Gossypol
C-77-I	$\text{CHCl}_3$	1.93	1.06	56.2 <sup>a</sup>	0.858
	$\text{CHCl}_3$	2.07	1.07	56.2 <sup>a</sup>	0.858
C-77-VII	$\text{CHCl}_3$	1.59	0.90	64.8	0.991
	$(\text{C}_2\text{H}_5)_2\text{O}$	2.21	1.13	65.5	1.00
C-77-IX	$\text{CHCl}_3$	2.00	1.13	66.3	1.01
	$(\text{C}_2\text{H}_5)_2\text{O}$	2.14	1.18	66.3	1.01
205e <sup>b</sup>	$\text{CHCl}_3$	1.64	1.10	22.5	0.344
	$(\text{C}_2\text{H}_5)_2\text{O}$	1.71	0.95	22.5	0.344
C-C-77-VIIa <sup>c</sup>	$\text{CHCl}_3$	1.23	0.92	15.1	0.235
PC-7 <sup>c</sup>	$\text{CHCl}_3$	1.78	1.04	32.1	0.481
205a <sup>b</sup>	$\text{CHCl}_3$	1.98	1.15	84.2	1.29
C-9 <sup>c</sup>	$\text{CHCl}_3$	1.94	1.12	70.0	1.07
205b	$\text{CHCl}_3$	2.04	1.17	67.2	1.03
C-78	$\text{CHCl}_3$	2.29	1.19	45.2	0.681

<sup>a</sup> Duplicate tests of same extract.<sup>b</sup> Raw cottonseeds contained considerable quantities of orange-colored pigment, so that chloroform solutions were treated with concentrated hydrochloric acid prior to reaction with antimony trichloride.<sup>c</sup> Hydraulic-pressed meals treated as in <sup>b</sup>.

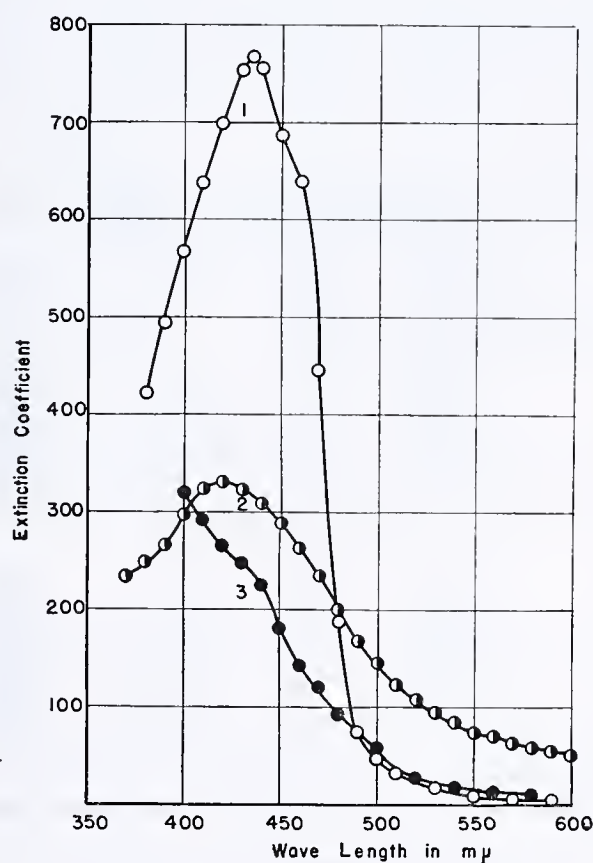
Fortunately, whether because of compensation or a slower development of interference at this point, within 10 minutes of mixing the antimony trichloride solution with the extract the absorption at 520  $m\mu$  increases to a value which remains constant for a considerable period of time, ranging from 24 hours for some extracts to a minimum of 30 minutes for the least stable of a series of 117 extracts of 64 different raw cottonseeds and meals examined. Therefore, the value of the extinction at 520  $m\mu$  of the antimony trichloride reaction products with chloroform solutions of ether or chloroform extracts of cottonseed, or with acid-treated chloroform solutions of ether or chloroform extracts of hydraulic-pressed cottonseed meals determined within 10 to 30 minutes after the reagents are mixed, is a true measure of the gossypol content of the extract.

As is shown in Table III, the addition of measured amounts of pure gossypol to cottonseed extracts produces increases in the extinction at 520  $m\mu$  of the reaction product with antimony trichloride which are quantitatively proportional to the amount of pure gossypol added. The values shown in Table III establish the precision of the method. When conducted as described, the antimony trichloride reaction method permits the determination of the gossypol content of ether and chloroform extracts of cottonseed and of hydraulic-pressed cottonseed meals with a duplicability within  $\pm 1\%$  of the total gossypol concentration of the extracts. This is the limit of precision of the spectrophotometer as it is used in the test.

Absorption spectra curves of antimony trichloride reaction products with cottonseed oils or Skellysolve F extracts differ markedly from that of the gossypol-antimony trichloride reaction product. As shown in curve 3 of Figure 3, when the antimony trichloride reaction product of such an extract has stood for some time, well-defined absorption maxima at 380, 450, and 490  $m\mu$  develop. Treatment of chloroform solutions of the oils or Skellysolve F extracts with concentrated hydrochloric acid prior to reaction with antimony trichloride causes the formation of pink reaction products. The absorption spectra curves no longer exhibit a maximum at 450  $m\mu$  and absorption at 510 to 540  $m\mu$  is increased, but absorption at 390 and 490  $m\mu$  remains high, so that the absorption curves only very slightly resemble the gossypol-antimony trichloride absorption curve. These observations indicate the presence of the orange-colored pigment in cottonseed oils and Skellysolve F extracts. They indicate further, however, that the orange-colored pigment occurs in such relatively small amounts that even when it has been converted to gossypol by the action of hydrochloric acid, the gossypol-antimony trichloride reaction is masked by the reaction of the preponderant interfering pigments. Chloroform and ether extracts of nondefatted cottonseed evidently contain some of this interfering substance, but in much lower relative concentrations, so that its only effect is to reduce the stability of the spectrophotometric test.

Since it has been possible to establish the specificity of the antimony trichloride reaction for gossypol in extracts prepared from 64 different samples of raw cottonseed and hydraulic-pressed cottonseed meal, the validity of the method can be considered to be established beyond reasonable doubt. Consequently, it appears superfluous to make a systematic comparison of the present method with other methods for the determination of gossypol.

The spectrophotometric method for the determination of gossypol proposed by Lyman, Holland, and Hale (8) suffers from the disadvantage that the dianilinogossypol absorption curve on which the method is based exhibits only one maximum in the visible region. Because of this limitation, the authors are able to state concerning the specificity of their method only that "there appears to be no source of error due to other pigments present in cottonseed meal", and "if there are other substances besides gossypol in cottonseed meal which give color with aniline, these substances must be closely related to gossypol". When various cottonseed extracts are treated with the carbonyl reagent, dinitrophenylhydrazine, the absorption spectra of the reaction mixtures indicate that carbonyl compounds other than

**Figure 4. Absorption Curves of 2,4-Dinitrophenylhydrazine Reaction Products with Cottonseed Components**

1. 2,4-Dinitrophenylhydrazone of orange-colored pigment of cottonseed
2. 2,4-Dinitrophenylhydrazone of gossypol
3. 2,4-Dinitrophenylhydrazone reaction product with chloroform extract of cottonseed

**Table III. Determination of Gossypol Added to Cottonseed Extracts**

Sample of Seed or Meal Extracted	Gossypol in Extract G./100 ml.	Pure Gossypol Added to Extract G./100 ml.	Total Gossypol in Final Mixture G./100 ml.	Gossypol Found G./100 ml.	Recovery of Gossypol %
202a	0.00208	0.00416	0.00624	0.00631	101.0
PC-1	0.00516	0.00074	0.00590	0.00599	101.5
	0.00387	0.00147	0.00534	0.00542	101.4
	0.00184	0.00323	0.00507	0.00508	100.2
C-101	0.00184	0.00586	0.00770	0.00760	98.7
	0.00368	0.00439	0.00807	0.00764	94.7
	0.00460	0.00366	0.00826	0.00812	98.3



gossypol and the orange-colored pigment occur in the cottonseed extracts. If these carbonyl compounds also react with aniline, as predicted by Lyman, Holland, and Hale (8), they will interfere in the aniline-spectrophotometric method for gossypol whenever they occur in cottonseed extracts. Consequently, no direct comparison of the two spectrophotometric methods was made.

Experience with the aniline precipitation method as modified by Halverson and Smith (6) and Smith (13) was similar to that recently reported by Lyman, Holland, and Hale (8) in that duplicate analyses were obtained with difficulty. No attempt was made to confirm their observation (8) that the dianilinogossypol precipitates were frequently impure. On the other hand, the antimony trichloride method nearly always indicated a higher gossypol concentration in a given extract than the aniline precipitation method. This occurred even when the extracts contained no detectable amounts of the orange-colored pigment. In such cases it seems most logical to conclude that the observed discrepancies are due to incomplete precipitation of dianilinogossypol from the extracts. According to a recent report (10), the addition of an ether solution of aniline to an ether solution of gossypol results in the precipitation of a mixture of dianilino- and tetraanilinogossypol. If a similar reaction should occur when aniline is added to ether extracts of cottonseed or cottonseed meal, the gossypol content of the extract should not be calculated on the assumption that the precipitate is dianilinogossypol.

Table IV. Time for Complete Extraction of Gossypol from Cottonseed and Cottonseed Meal

Sample	Extraction Solvent	Time, Hours	E at 520 m $\mu$ per Gram	Per Cent Gossypol
Cottonseed containing negligible amounts of the orange-colored pigment				
17a	CHCl <sub>3</sub>	0.5	13.5	0.206
	CHCl <sub>3</sub>	1	23.1	0.352
	CHCl <sub>3</sub>	2	34.6	0.528
	CHCl <sub>3</sub>	2	34.2	0.523
	CHCl <sub>3</sub>	24	36.1	0.552
C-77-VII	CHCl <sub>3</sub>	2	64.5	0.975
	CHCl <sub>3</sub>	24	63.0	0.963
	(C <sub>2</sub> H <sub>5</sub> ) <sub>2</sub> O	2	65.5	1.00
Cottonseed and hydraulic-pressed meal containing considerable amounts of orange-colored pigment <sup>a</sup>				
PC-77	(C <sub>2</sub> H <sub>5</sub> ) <sub>2</sub> O	2	11.7	0.179
	CHCl <sub>3</sub>	2	12.4	0.189
	CHCl <sub>3</sub>	24	15.1	0.235
	CHCl <sub>3</sub>	48	15.1	0.235
205e	(C <sub>2</sub> H <sub>5</sub> ) <sub>2</sub> O	2	16.8	0.257
	CHCl <sub>3</sub>	2	17.3	0.264
	CHCl <sub>3</sub>	24	22.5	0.344
	(C <sub>2</sub> H <sub>5</sub> ) <sub>2</sub> O	24	22.5	0.344
	CHCl <sub>3</sub>	48	22.8	0.348

<sup>a</sup> All CHCl<sub>3</sub> solutions of extracts treated with concentrated HCl prior to reaction with SbCl<sub>3</sub>.

#### EXTRACTION OF GOSSYPOL FROM COTTONSEED

With a rapid and accurate method available for the determination of gossypol in cottonseed extracts, the accurate determination of gossypol in cottonseed requires only a reliable and convenient method for extracting gossypol from the seed.

It has been reported that both the duration of extraction and type of extraction apparatus, as well as the moisture content of the seed or hydraulic-pressed meal, affect the amount of gossypol extracted. Most investigators (5-8, 11-13) recommend exhaustive extraction, usually in an apparatus of the reflux type which is designed for thorough rinsing of the substance from which soluble material is extracted. Apparatus of the reflux type must be used when the seed or meal is to be freed entirely from gossypol, or when, as is the case in the use of gravimetric methods, a large amount of gossypol is required for the determination. On the other hand, a simple equilibration can be used effectively when the concentration of extractable gossypol in the seed or meal is to be determined by means of the sensitive spectrophotometric method. The conditions for adequate

Table V. Equivalence of Equilibration and Butt Extraction with Ether

Cotton-seed Sample <sup>a</sup>	Kind of Extraction	Time of Extraction, Hours	R <sub>a</sub>	R <sub>b</sub>	E at 520 m $\mu$ per Gram Seed	Per Cent Gossypol
C-77-VIII	Equilibration	2	1.97	1.03	61.9	0.945
	Equilibration	24	2.15	1.16	64.6	0.986
	Equilibration	72	1.90	1.04	62.8	0.960
	Butt	24	2.05	1.23	68.8	1.05
	Butt	72	1.95	1.03	68.8	1.05
C-77-IX	Equilibration	24	2.03	1.16	60.3	0.922
	Butt	24	2.03	1.19	62.9	0.960
	Equilibration <sup>b</sup>	2	2.00	1.13	66.3	1.012
	Equilibration	2	2.14	1.18	66.3	1.012

<sup>a</sup> Both series of cottonseed samples contained negligible amounts of orange-colored pigment.

<sup>b</sup> Solvent of equilibration was chloroform.

equilibration can be ascertained by determining what volume of solvent and what contact time must be employed, so that an increase in the proportion of solvent or in the time of contact produces no increase in the concentration of gossypol in solution. An aliquot portion of an extract obtained under these conditions can be used directly in the spectrophotometric method for determining the concentration of extractable gossypol in the seed.

As is shown in Table IV, complete extraction of gossypol from ground cottonseed meals or hydraulic-pressed meal by equilibration with chloroform requires 2 hours when the seed contains negligible amounts of the orange-colored pigment, and 24 hours when the seed or meal contains a considerable concentration of this pigment.

That diethyl ether and chloroform extract equal amounts of the pigments is demonstrated in Tables II, IV, VI, and VIII. Murty, Murty, and Seshadri (9) also have reported the equivalence of ether and chloroform for the extraction of gossypol.

In order to confirm the equivalence of simple equilibration and exhaustive extraction, direct comparisons of extracts of replicate samples of the same seeds were made (Table V).

The results of experiments to determine the effect of moisture on the extraction of free gossypol by chloroform and ether are shown in Table VI. These data show, in agreement with the recent report of Murty, Murty, and Seshadri (9), that moisture does not play an important role unless it is very low, as in the case of desiccated seed, or so high as to interfere with the "wetting" of the seed by chloroform. Halverson and Smith (7) and Lyman, Holland, and Hale (8) observed that continued extraction of wet hydraulic-pressed cottonseed meal by wet ether at elevated temperatures gives increased amounts of gossypol. They attributed their results to the absence of a sharp boundary between "free" and "bound" gossypol in cottonseed meal. In view of the results obtained with raw cottonseed, it is more probable that the extraction methods employed by these investigators cause the liberation of some of the bound gossypol. Consequently, for the extraction of free gossypol from cottonseed meal the present authors have employed only the mild conditions of extraction found adequate for the extraction of free gossypol from raw cottonseed.

It is apparent from Table VII that proportions of chloroform to seed varying from 25 to 125 ml. per gram afford complete extraction of gossypol. Consequently, the spectrophotometric method can be used for accurate determination of the gossypol content of seeds of widely varying gossypol content.

In contrast with the consistent results obtained by the equilibration of ground cottonseed kernels and of hydraulic-pressed meal with chloroform and ether shown in the preceding table and in Table VIII, very erratic results were obtained when the seed was first defatted by extraction with Skellysolve F. As is shown in Table VIII, extraction of gossypol from defatted seed was erratic regardless of the nature of the solvent or of the moisture content of the seed. Moreover, despite published reports that no gossypol can be detected in Skellysolve F extract



(3, 7, 14), it was found, as is shown in Table VIII, that the indicated gossypol content of Skellysolve F-defatted seed is almost invariably less than that of the nondefatted seed. In view of these facts, it is apparent that accurate gossypol determinations can be obtained only by the direct extraction of nondefatted seed with ether or chloroform.

#### PROCEDURE FOR DETERMINATION OF EXTRACTABLE GOSSYPOL OF COTTONSEED AND COTTONSEED MEAL

**REAGENTS.** The chloroform and acetic anhydride should be C.P. grade and the antimony trichloride should be anhydrous, C.P. grade.

The saturated chloroform solution of antimony trichloride is prepared as follows: Wash about 30 grams of finely ground antimony trichloride with a small volume of chloroform. Add 100 ml. of chloroform to the washed crystals, warm the suspension, shake it vigorously, and allow it to cool to room temperature.

The gossypol used for the standardization should be purified as previously described (1, 2).

**PROCEDURE.** A measured volume of chloroform is added to a weighed sample of ground cottonseed kernels or meal. (For the extraction of raw cottonseed of the usual range of gossypol content, 25 ml. of chloroform to 0.25 gram of ground kernels produce an extract which can be used directly in the antimony trichloride test without dilution. For most hydraulic-pressed meals a larger proportion of meal to chloroform must be used.) The flask is stoppered and the mixture is allowed to stand, with occasional shaking, for 24 hours. A sample of the extract is withdrawn from the equilibration mixture in a manner which prevents evaporation and filters the extract—e.g., by covering the tip of a pipet with cotton or by attaching an inverted sintered-glass suction funnel to the end of a pipet. The sample is then shaken vigorously with concentrated hydrochloric acid, about 10 drops per 5 ml. of extract, and the mixture is allowed to stand for 24 hours.

One milliliter of the extract is transferred to a glass-stoppered absorption cell by means of a pipet. One drop of acetic anhydride and 5 ml. of a saturated chloroform solution of antimony trichloride are introduced into the absorption cell and the mixture is thoroughly agitated.

Within 10 to 40 minutes after the reagents are mixed, the transmission of the test solution at 520  $m\mu$  is read against that of a blank consisting of 1 ml. of chloroform, 1 drop of acetic anhydride, and 5 ml. of a saturated chloroform solution of antimony trichloride.

The concentration of gossypol in the original seed or meal is calculated by means of the following equation:

$$\% \text{ gossypol} = \frac{\log I_0/I \times V/W}{E^{1\%} \text{ gossypol}}$$

$\log I_0/I$  is the extinction at 520  $m\mu$  of the gossypol-antimony trichloride test solution.  $I_0$  is the transmission of the antimony trichloride reagent blank at 520  $m\mu$ .  $I$  is the transmission of the gossypol-antimony trichloride test solution at 520  $m\mu$ .  $V$  is the volume of solvent used in the extraction of the seed.  $W$  is the weight of cottonseed extracted.  $E^{1\%}$  gossypol is the extinction coefficient at 510 to 520  $m\mu$ , as previously defined, calculated for 1% gossypol in the original solution before reaction with antimony trichloride. With the absorption cells used in the Coleman double monochromator spectrophotometer  $E^{1\%}_{1.27 \text{ cm.}} = 65.5 \pm 1.9$ .

Table VI. Effect of Moisture on Extraction of Gossypol from Cottonseed

Treatment of Ground Meats <sup>a</sup>	Solvent	E at 520 $m\mu$ per Gram	Per Cent Gossypol
None	CHCl <sub>3</sub>	66.3	1.013
H <sub>2</sub> O added	CHCl <sub>3</sub>	61.0	0.932
None	(C <sub>2</sub> H <sub>5</sub> ) <sub>2</sub> O	66.3	1.013
H <sub>2</sub> O added	(C <sub>2</sub> H <sub>5</sub> ) <sub>2</sub> O	70.4 <sup>b</sup>	1.073
None	CHCl <sub>3</sub>	64.8	0.990
Dried <sup>c</sup>	CHCl <sub>3</sub>	11.3	0.107
Dried <sup>c</sup>	Wet (C <sub>2</sub> H <sub>5</sub> ) <sub>2</sub> O <sup>d</sup>	69.5	1.061
Dried <sup>c</sup>	Wet (C <sub>2</sub> H <sub>5</sub> ) <sub>2</sub> O <sup>d</sup>	65.3	0.998
Dried <sup>c</sup>	Wet (C <sub>2</sub> H <sub>5</sub> ) <sub>2</sub> O <sup>d</sup>	67.0	1.022
Dried <sup>e</sup>	(C <sub>2</sub> H <sub>5</sub> ) <sub>2</sub> O	62.9	0.961
Dried <sup>e</sup>	CHCl <sub>3</sub>	62.9	0.961
Wet <sup>f</sup>	(C <sub>2</sub> H <sub>5</sub> ) <sub>2</sub> O	65.2	0.996

<sup>a</sup> Original meats contained 8.51% moisture.

<sup>b</sup> Sediment observed in filtered extract.

<sup>c</sup> Dried in desiccator to 3.12% moisture.

<sup>d</sup> Ether contained 1% H<sub>2</sub>O and 2.5% C<sub>2</sub>H<sub>5</sub>OH.

<sup>e</sup> Dried as in <sup>c</sup>, then exposed to moist air for 24 hours. Final meats contained 7.57% moisture.

<sup>f</sup> Dried as in <sup>c</sup>, then moistened with 2 drops of H<sub>2</sub>O per 0.25 gram of ground meats.

Table VII. Effect of Variation in Ratio of Volume of Extraction Solvent to Weight of Cottonseed

Cottonseed Sample	Ml. of CHCl <sub>3</sub> per Gram of Meats	E at 520 $m\mu$ per Gram	Per Cent Gossypol
C-77-VIII	25	62.0	0.947
	50	61.0	0.932
	125	62.0	0.947
C-77-VII	25	63.6	0.971
	50	63.0	0.962
	100	64.8	0.990
C-77-IX	25	66.3	1.01
	50	64.8	0.990
	100	62.8	0.948
205e	100	22.9	0.350

Table VIII. Extraction of Gossypol from Defatted and Nondefatted Cottonseed Meats

Cottonseed Sample	Treatment of Ground Meats Prior to Equilibration	Per Cent Moisture	Equilibration Solvent	R <sub>a</sub>	R <sub>b</sub>	E at 520 $m\mu$ per Gram <sup>a</sup>	Per Cent Gossypol
C-77-VII	None	8.70	(C <sub>2</sub> H <sub>5</sub> ) <sub>2</sub> O	2.21	1.13	65.5	1.00
	None	8.70	CHCl <sub>3</sub>	1.59	0.90	64.8	0.991
	None	8.70	CHCl <sub>3</sub>	1.90	1.01	64.5	0.985
	None	8.70	CHCl <sub>3</sub>	1.92	1.10	64.5	0.985
	Defatted	12.29	CHCl <sub>3</sub>	1.57	1.01	30.6	0.467
	Defatted	12.29	CHCl <sub>3</sub>	1.70	0.97	58.5	0.893
	Defatted	12.29	CHCl <sub>3</sub>	1.83	0.98	39.1	0.597
	Defatted	12.29	CHCl <sub>3</sub>	2.14	1.19	39.2	0.598
	Defatted	<sup>b</sup>	CHCl <sub>3</sub>	1.86	1.19	36.1	0.552
	Defatted	12.29	CHCl <sub>3</sub>	1.81	1.13	43.2	0.660
	Defatted	12.29	(C <sub>2</sub> H <sub>5</sub> ) <sub>2</sub> O	2.10	1.11	39.2	0.598
	Defatted	12.29	(C <sub>2</sub> H <sub>5</sub> ) <sub>2</sub> O <sup>d</sup>	2.30	1.12	38.1	0.582
C-77-VIII	None	7.17	CHCl <sub>3</sub>	2.04	1.10	61.0	0.932
	None	7.17	CHCl <sub>3</sub>	1.85	0.99	62.0	0.947
	None	7.17	CHCl <sub>3</sub>	1.94	1.03	61.0	0.932
	Defatted	.....	CHCl <sub>3</sub>	1.97	1.03	61.9	0.945
	Defatted	.....	CHCl <sub>3</sub>	1.89	1.09	46.8	0.716
	Defatted	.....	CHCl <sub>3</sub>	1.81	1.07	46.2	0.710
C-77-IX	None	8.51	CHCl <sub>3</sub>	2.00	1.13	66.3	1.01
	None	8.51	(C <sub>2</sub> H <sub>5</sub> ) <sub>2</sub> O	2.14	1.18	66.3	1.01
	None	8.51	CHCl <sub>3</sub>	2.07	1.10	64.8	0.991
	Defatted	12.50	CHCl <sub>3</sub>	1.79	1.07	42.5	0.648
C-105b	None	.....	CHCl <sub>3</sub>	2.04	1.17	67.2	1.027
	Defatted	.....	CHCl <sub>3</sub>	1.82	1.09	41.7	0.637
C-78	None	.....	CHCl <sub>3</sub>	2.29	1.19	45.2	0.690
	Defatted	.....	CHCl <sub>3</sub>	1.80	1.13	23.4	0.357

<sup>a</sup> Calculated on basis of meats before defatting.

<sup>b</sup> Five drops H<sub>2</sub>O added to 0.5 gram of defatted meats.

<sup>c</sup> Five drops refined cottonseed oil added to 0.5 gram of defatted meats

<sup>d</sup> Ether containing 1% H<sub>2</sub>O, 2.5% C<sub>2</sub>H<sub>5</sub>OH.

#### SUMMARY

The reaction of gossypol with antimony trichloride in chloroform produces a soluble red product having a characteristic absorption curve in the visible and near ultraviolet region of the spectrum. The absorption curve exhibits a broad, stable maximum at 510 to 520  $m\mu$ . The extinction at this absorption maximum is proportional to the concentration of gossypol.

The antimony trichloride reaction is specific for gossypol in ether and chloroform extracts of cottonseed and the reaction product is sufficiently stable to permit the accurate determination of the gossypol concentration of such extracts by means of the spectrophotometer.

The gossypol-antimony trichloride reaction has been used as a means for determining the optimum conditions for the extraction of free gossypol from cottonseed. Chloroform and ether extract equal amounts of gossypol. Equilibration of meats and solvent for 24 hours is adequate for complete solution of extractable gossypol. Moisture is an important factor in the extraction of gossypol only in the case of very dry seeds. The proportions of solvent to meats may be varied within wide limits.

A rapid, direct method for the determination of "free" gossypol in cottonseed has been reported in which the gossypol is extracted by equilibrating ground cottonseed and chloroform. The extract is treated with concentrated hydrochloric acid and the antimony trichloride reaction is applied directly to the treated extract.

The method has been shown to be applicable to the determination of the free gossypol content of hydraulic-pressed meal in which gossypol occurs to a large extent in the form of an orange-



colored pigment which is not precipitated by aniline but is readily converted to gossypol.

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# Determination of Thiamine by the Thiochrome Method

## Effects of Temperature and Dissolved Oxygen on Fluorescence of Quinine Standard and of Thiochrome

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The effect of dissolved oxygen and changes in temperature upon the quinine standard used in the thiochrome reaction and upon thiochrome solutions is large enough to warrant an attempt to control these variables. Temperature effects can be minimized by the use of a water bath to keep the quinine at a standard temperature. The effect probably does not alter the thiochrome fluorescence very much if the room temperature does not vary greatly. Oxygen effects can be minimized by controlling the temperature of the quinine so that no dissolved air is lost, or by the use of glass standards. Because of the shaking operation the oxygen content of thiochrome solutions is probably a constant factor. Since several types of instruments used to measure fluorescence will gradually heat up the cuvette chamber, the quinine standard should not be left in the instruments.

IT HAS long been apparent to many analysts that the thiochrome method of assaying the thiamine content of biological and other products is occasionally subject to unexplained sources or error that appear and disappear in an erratic manner. Usually these errors limit the accuracy of the method to from  $\pm 5\%$  to  $\pm 10\%$  (5), but they may be considerably larger, as has occasionally been observed in this laboratory. While engaged in an attempt to run down some of these sources of error the authors became suspicious of the accuracy of their quinine standard. The order in which samples of thiamine were oxidized and read against the quinine standards appeared to affect the results. If a sample of material was assayed twice on any given day, and if several hours elapsed between the two oxidations, the last result was the higher if the same quinine standard was used for both oxidations. This phenomenon made it appear as if the quinine exhibited less fluorescence the longer it was used on any given day. The authors had been using fresh daily aliquots of the standard, kept at about 6° C. when not in use.

According to Vavilov's equations (6) the fluorescence of a substance in solution is a function of the absolute temperature, other variables being constant. Vavilov also demonstrates the quenching of fluorescence by foreign molecules in the fluorescing solution. He divides quenching into two types (13): quenching by redistribution of the absorbed radiant energy among the

degrees of freedom of the fluorescence molecule itself and collisions of the second type, which may or may not involve a chemical reaction. A consideration of Vavilov's work, together with the observations mentioned above, led to the conclusion that the possible effects of temperature and quenchers on the fluorescence of quinine and thiochrome should be investigated.

Quenching by redistribution of energy among the molecules of the fluorophor, if it occurs in the quinine standards and in concentrations of thiochrome usually used in the thiochrome method, would appear to be a constant factor, at least for quinine. The only possibilities for quenching by foreign molecules lie in the presence of dissolved atmospheric gases in both quinine and thiochrome solutions, of potassium chloride, sodium sulfate, water alkali, or ferricyanide in the thiochrome solution, and of sulfuric acid in the quinine solution. The quenching effect of dissolved oxygen was noted by Weil-Malherbe and Weiss (14), who found that oxygen at one atmosphere of pressure quenched thiochrome fluorescence in isobutyl alcohol 27.5% and quinine sulfate fluorescence in 0.1 N sulfuric acid 17.5% when both substances were examined in a concentration of 10 mg. per liter. The quenching ability of electrolytes has been intensively studied by Stoughton and Rollefson (9, 10, 11), who found the chloride ion to be a strong quencher for quinine.

The fluorescence of quinine is profoundly affected by the presence of acid. It changes in color from blue to violet from pH 3.8 to 4.5 (4) and decreases to zero at pH 9 (3). At pH 2 the fluorescence is proportional to the concentration of quinine and at pH 3 is a logarithmic function of concentration (2). Changes in pH change the fluorescence spectrum of quinine sulfate (7). It has also been reported (1) that the spectral line of fluorophors is changed by changes in temperature.

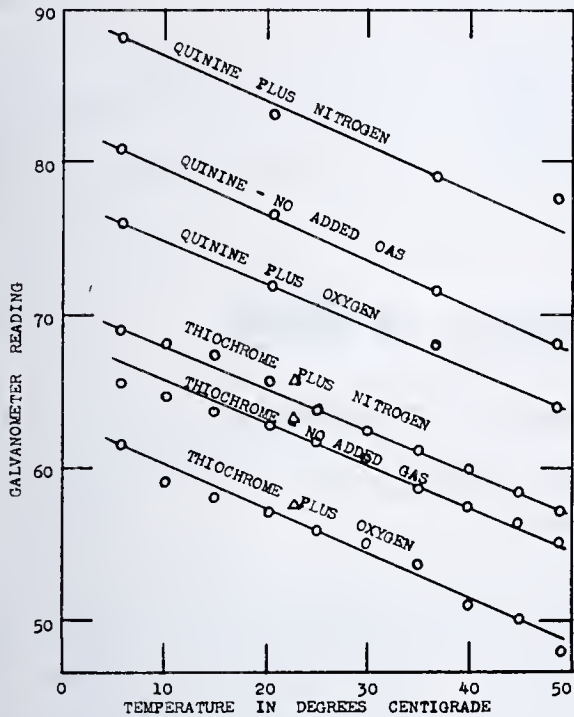
## PROCEDURE

Three aliquots of the quinine standard (0.3 microgram per ml. of 0.1 N sulfuric acid) and three aliquots of thiamine oxidized to thiochrome (1.0 microgram thiamine aliquots oxidized to thiochrome and dissolved in 18-ml. aliquots of isobutanol) were placed in glass cuvettes. The cuvettes were stoppered and the thiochrome cuvettes were covered with metal covers to exclude light. Two sets of each series were connected to manifolds. Oxygen was blown through one set for 15 minutes and nitrogen was blown through the other set for the same length of time. Volume changes from the blowing were prevented by first saturating the gases with water vapor (quinine samples) or isobutyl alcohol (thiochrome samples). The third set of each series was left intact

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(no added gas). All the cuvettes were placed in a water bath at about 6° C. Another quinine standard was heated to 35° C. in a water bath and bubbles of expelled air were removed. It was maintained at this temperature by the water bath and used as a reference standard. The series of quinine and thiochrome solutions were read at various temperatures from 6° to 50° C. and the galvanometer readings they produced in a fluorometer were recorded.



**Figure 1. Quenching Effect of Temperature upon Quinine Sulfate and Thiochrome**  
0.3  $\mu$ g. of quinine sulfate per ml. of 0.1N  $H_2SO_4$ . 1.0  $\mu$ g. of thiamine oxidized to thiochrome and dissolved in 18 ml. of isobutanol

The results are shown in Figure 1. Clearly, within the temperature range studied, the fluorescence as measured by galvanometer deflection is a linear function of temperature, and oxygen is a strong quenching agent for both thiochrome and quinine. As the solutions warmed up from 6° C. to a room temperature of 25° C. the galvanometer deflection varied about 5 units. Since it is customary to keep quinine solutions under refrigeration, it is clear that such solutions should be warmed to room temperature before use.

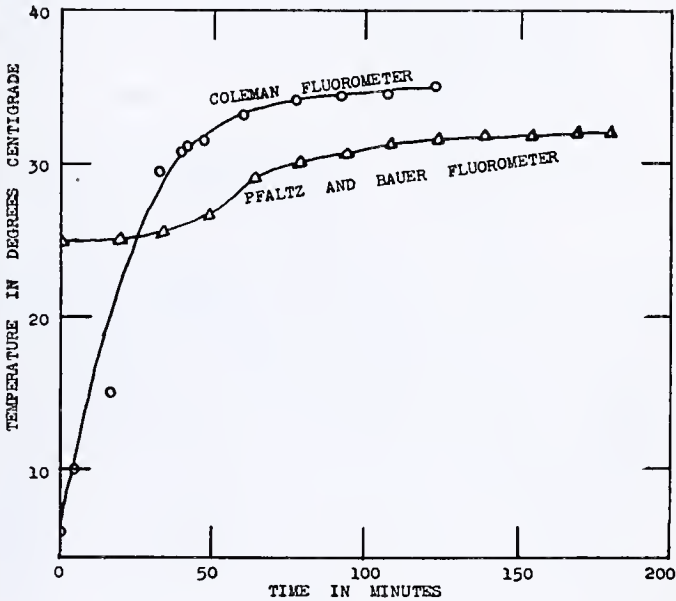
To make sure that the effects noticed were not caused by decomposition, particularly by decomposition of thiochrome, the solutions were cooled from 50° to 22° C. and readings were again taken on the thiochrome solutions. These readings, shown by the triangle points in Figure 1, indicate that no decomposition of thiochrome took place. A fresh sample of quinine was taken and read against the standard held at 35° C., then heated to 50° C., cooled, and read again. Both readings were 90 galvanometer units. Then the fresh sample was placed in the fluorometer and allowed to stand in the ultraviolet beam for 45 minutes. At the end of that time it read 87.5 galvanometer units. Apparently quinine is stable to heat up to 50° C. and to the light from the authors' fluorometer.

In this laboratory it has been customary to test the fluorometer with the quinine standard both before and after each reading. During the interval between the readings of successive samples (during the oxidations) the quinine is allowed to stay in the cuvette chamber, as the instrument (a Coleman Model 12 photo-fluorometer) is provided with a shutter, so that the light can be shut off from the cuvette chamber. It was decided to find out if the quinine standard was warmed by the instrument during the 2-hour period it takes to oxidize and read the daily run.

A sample of quinine was taken from the refrigerator at about 6° C. and placed in the instrument. The instrument was turned on and temperature readings were taken at short intervals for 2 hours. The resulting curve is shown in Figure 2. In 2 hours the solution warmed up to 35° C. A similar curve was run on another type of fluorometer in which the cuvette chamber is separate from the light source. This sample was taken at room temperature and the curve is also shown in Figure 2.

A comparison of Figure 2 with Figure 1 makes it clear that the quinine standard should not be left in either type of instrument. One instrument in a 2-hour period can raise the temperature of the quinine standard to 35° C.

If the quinine standard is taken from a refrigerator at 6° C. and used immediately, setting the instrument at a galvanometer reading of 80 with it, and if it is allowed to warm up to 35° C. in the machine while in use, the variable resistances in the fluorometer will have to be changed in an amount corresponding to a galvanometer deflection of 8.75 units to keep the quinine reading at 80 units. This is obvious from Figure 1 (curve for quinine, no added gas), since a temperature rise of 6° to 35° C. will cause the galvanometer deflection of the quinine to drop from 80.75 to 72.0 units, a difference of 8.75 units. This can cause errors of 10.9% in the determination of an unknown. Since the more probable variations in temperature of the quinine solution would perhaps involve only a 10° variation, the more common errors caused by temperature changes may well lie in the neighborhood of 3 galvanometer units if the galvanometer readings are made in the neighborhood of 80 units. This would introduce errors of the order of 4%. No errors will be introduced from temperature variations, provided both quinine and thiochrome solutions are read at the same temperature. It is apparent that for best results both the quinine standard and thiochrome solutions should be maintained at the same constant temperature within  $\pm 2^\circ$  or  $3^\circ$  C.



**Figure 2. Heating of Quinine Sulfate Solution in Cuvette by Fluorometers**

The effect of dissolved oxygen also depends upon the temperature. This effect is probably more or less constant in the case of the thiochrome because of the shaking operation when the thiochrome is extracted with isobutanol, and nonexistent in the quinine standard in cases where the temperature is controlled in such a way that bubbles of dissolved gases are not expelled. It could be eliminated by the use of glass standards such as those described by Vastagh and Szegho (12) and Lowenstein (8). Elimination of temperature changes caused by placing of solutions on or near open windows, radiators, or steam pipes, and cooling of isobutanol to room temperature after distillation are among the considerations suggested by the data presented.

To test the effect of potassium chloride on the thiochrome fluorescence, samples were run both with and without the addition of potassium chloride. The resulting temperature-



fluorescence curves were identical with those in Figure 1 for thiochrome with no added gas. Identical curves were also obtained when a sample of thiamine was oxidized with twice the usual amount of alkaline ferricyanide, indicating that reagent has no appreciable quenching effect beyond the amounts necessary for oxidation.

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## Photometric Determination of Silica In Condensed Steam in Presence of Phosphates

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IN RECENT years much difficulty has been experienced in steam power plants because of silica deposition on turbine blades. As part of the study of the cause of this difficulty, it became necessary to have available a rapid method of analysis for small amounts of silica (as low as 0.05 p.p.m.) in condensed steam. Since phosphate might also be present in the steam, it was necessary to determine the silica in the presence of phosphate.

Kahler (2) used a method involving measuring the molybdenum blue color developed by reducing the yellow silicomolybdate complex with sodium sulfite at a suitable pH (pH 2.4 to 2.7 before reduction). The proper adjustment of the pH reduced the interference of phosphate in the concentrations he studied (silica between 5 and 40 p.p.m.). Kahler points out that as the acidity decreases above 2.7, the effect of the phosphate becomes negligible, but the color development when silica is present requires more time and is accompanied by considerable color progression. In order to increase the sensitivity of this method for low silica contents, it was deemed advisable to have a pH of between 2.2 and 2.4 before adding the sulfite and to change the concentrations of the solutions added, to secure a lower dilution effect. Kahler used 10 ml. of sample and added 20 ml. of reagent solutions, thus having a final volume three times his sample. The modified reagents used were: hydrochloric acid reagent, 55 ml. of 38% grade (1.19 specific gravity) plus 900 ml. of distilled water. Ammonium molybdate reagent, 10 grams of ammonium molybdate (reagent grade) plus 800 ml. of distilled water. Sodium sulfite reagent, 135 grams of sodium sulfite (anhydrous reagent grade) plus 800 ml. of distilled water. Sodium silicate solution, 10.0 mg. as silica per liter. Fifty milliliters of sample were used for analysis, and 5 ml. of hydrochloric reagent, 10 ml. of ammonium molybdate reagent, and 10 ml. of sodium sulfite were added, giving a final volume of 75 ml. or 1.5 times the sample.

It was realized that this procedure might not eliminate the effect of phosphate entirely, but it was thought best to try it, since the increased sensitivity was desirable and most of the samples being tested were free from phosphate.

#### APPARATUS

A Coleman spectrophotometer Model 11 was used for colorimetric comparison of the solutions. It was noticed that the time interval lapsing between addition of the ammonium molybdate reagent and of the sodium sulfite reagent, as well as the time elapsing between addition of the sodium sulfite reagent and taking of the reading, had a marked effect on the final reading. The time interval between addition of the ammonium molybdate reagent and the sodium sulfite reagent was standardized at 1

minute. A study was made of the effect of elapsed time between the addition of the sodium sulfite and the taking of the readings and Table I shows the effect of time on the reading. At the end of 10 minutes, the color was still changing; however, in order to save time, the readings were taken after a lapse of 5 minutes. The change was such that an error of 30 seconds in time would produce an error of 0.5%, which was considered well within the range of accuracy desired. The results shown in Figure 1 were obtained under these conditions of testing. A 40-mm. cell was used for testing and comparison was made at a wave length of 700 millimicrons.

The results obtained showed the method to be fairly sensitive for silica, but it was thought that a more sensitive method might be developed.

Schwartz (3) described a method for determining silica colorimetrically in the presence of phosphate, in which he made use of the yellow color developed by the yellow complex silicomolybdate and destroyed the phosphomolybdic acid complex by adding oxalic acid. Schwartz reagents and test procedure were as follows:

Hydrochloric acid reagent, 1 volume of concentrated acid to 1 volume of distilled water, 1 to 1.

Ammonium molybdate reagent, 10.0 grams of ammonium molybdate tetrahydrate per 100 ml. of distilled water.

Oxalic acid reagent, 10.0 grams of oxalic acid dihydrate per 100 ml. of distilled water.

Add and mix 1 ml. of hydrochloric acid solution and 2 ml. of ammonium molybdate solution in rapid succession to 50 ml. of sample. Wait 5 to 10 minutes for full color development, then add and mix 1.5 ml. of oxalic acid solution and determine color intensity.

Table I. Effect of Time on Color Development in Modified Kahler Method

(2 p.p.m. of SiO<sub>2</sub> in sample tested)

Elapsed Time after Addition of Sulfite Min.	Transmittance %
2	44.3
3	43.3
4	43.0
5	42.8
6	42.5
7	42.3
8	42.1
9	42.1
10	42.0



Figure 1 gives per cent transmittance at 410 millimicrons for various concentrations of silica using 19-mm. test tubes. A 5-minute interval was used after addition of the ammonium molybdate solution before addition of the oxalic acid solution. There was no change in transmittance reading in a period from 2 to 15 minutes after the addition of the oxalate solution.

This method eliminates the effect of time in taking the reading of transmittance; however, its sensitivity is about the same as the Kahler method.

A third method, suggested by Imhoff (1), was similar to the Schwartz method without the addition of the oxalic acid; however, the final solution was reduced by means of 1-amino-2-naphthol-4-sulfonic acid in sodium sulfite-bisulfite solution. This method would give high results in the presence of phosphate but would have a higher degree of sensitivity. By combining the complete Schwartz method with the last step in the Imhoff method, it was found possible to get a very sensitive method and to eliminate the interference of phosphate.

The reagents and test procedure finally used were as follows:

Hydrochloric acid reagent, 1 volume of concentrated acid to volume of distilled water, 1 to 1.

Ammonium molybdate reagent, 10.0 grams of ammonium molybdate tetrahydrate per 100 ml. of distilled water.

Oxalic acid reagent, 10.0 grams of oxalic acid dihydrate per 100 ml. of distilled water.

1-Amino-2-naphthol-4-sulfonic acid reagent, 30 grams of sodium bisulfite and 1 gram of sodium sulfite dissolved in 200 ml. of distilled water and 0.5 gram of 1-amino-2-naphthol-4-sulfonic acid added. The solution was heated slowly until the last reagent dissolved. Care should be taken not to heat solution too hot.

Add and mix 1 ml. of hydrochloric acid solution and 2 ml. of ammonium molybdate solution in rapid succession to 50 ml. of sample. Wait 5 minutes, then add and mix 1.5 ml. of oxalic acid solution, followed by 2 ml. of the 1-amino-2-naphthol-4-sulfonic acid. Determine silica color intensity with suitable instrument at a wave length of 700 millimicrons after 1 minute.

Figure 1 shows the per cent transmittance with a 40-mm. cell and 19-mm. test tubes. When phosphate in amounts equal to 50 p.p.m. was added to silica solutions no appreciable deviation from the curve on phosphate-free silica solutions was detected (Table II).

When 50 p.p.m. of phosphate was present and the oxalic acid was not added (Imhoff method), the transmittance of a blank without silica present was 1%; however, when a similar test was run with oxalic acid present, the transmittance was 100%. This shows that the phosphate had a marked effect on the Imhoff method, but that oxalic acid entirely eliminates the phosphate effect.

Table II shows that the accuracy of the method is about 0.02 p.p.m. of silica.

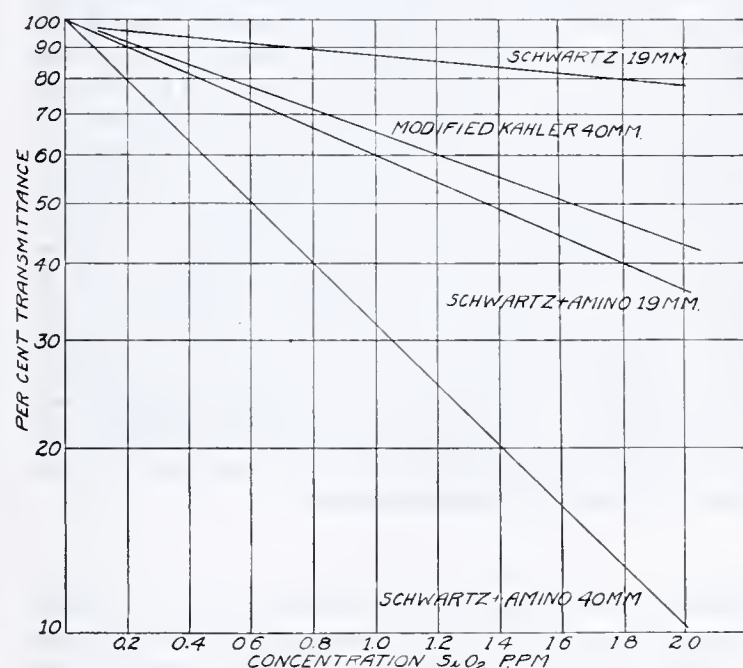


Figure 1

Table II. Effect of Phosphate on Transmittance

(Using Schwartz method with amino acid)

SiO <sub>2</sub> P.p.m.	PO <sub>4</sub> P.p.m.	Transmittance %
0.1	0	94.0
0.1	0	95.0
0.1	0	94.0
0.1	0	94.5
0.1	0	94.0
0.1	50	93.8
0.2	0	89.5
0.2	0	90.3
0.2	0	90.0
0.2	0	89.8
0.2	0	90.0
0.2	50	89.0
0.5	0	76.5
0.5	0	76.8
0.5	0	76.3
0.5	50	76.3
1.0	0	60.0
1.0	0	60.0
1.0	0	60.0
1.0	50	59.8
2.0	0	37.0
2.0	0	37.0
2.0	0	36.5
2.0	50	36.5

Tests conducted using standard silica solutions showed no effect on per cent transmittance with a time interval from 2 to 15 minutes after addition of the final reducing agent. With a time interval of only 3 minutes after addition of the ammonium molybdate, final color of less intensity was developed; however, as no change was determined when a time interval of 5 and 10 minutes was used, this time was set at 5 minutes.

Since reagents used add a small amount of color to the test solution and this might vary, owing to the possibility of dissolving silica from the glass containers used, a blank solution was prepared, using the same volume of silica-free distilled water as the test sample, to which the regular amount of reagents were added at the time the test sample was being tested. This blank was then put in the spectrophotometer, the reading adjusted to 100% transmittance, and the comparison made on the test sample. This eliminated the interference of the reagents or silica in the reagents. The blank usually read about 97% transmittance when compared with distilled water to which no reagents had been added.

This modification of the Schwartz method has a degree of accuracy of 0.01 p.p.m. in determining silica in amounts from 0.02 to 2.0 p.p.m., when a 50-ml. sample is used. If the silica is above 2 p.p.m., the Schwartz method would work better, since it would require less dilution. These tests have all been conducted on distilled water or condensed steam free from color or organic material. The effect of organic matter in the water has not been studied.

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PRESENTED before the Division of Water, Sewage, and Sanitation Chemistry at the 107th Meeting of the AMERICAN CHEMICAL SOCIETY, Cleveland, Ohio. Data from research conducted at the University of Illinois in cooperation with the Utilities Research Commission of Chicago and released by permission of both parties.



# Thiamine Determination

## Comparative Study of Yeast-Growth, Yeast-Fermentation, and Thiochrome Methods

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The yeast-growth, yeast-fermentation, and thiochrome methods for thiamine determination have been studied, with an attempted evaluation of certain modifications in the yeast methods. The thiochrome method appears to be a satisfactory means of determining the thiamine content of various types of natural and processed materials. Judging from values obtained by the other methods, thiochrome values may be somewhat low, owing to the presence in some extracts of substances which interfere with the quantitative adsorption of the vitamin on Decalso. The yeast-growth method gives somewhat higher values than the other methods. In the case of processed materials, they are so high as to be without merit. The specificity of this method can be increased by use of an adsorption technique which permits separation of thiamine from other materials active in yeast growth. A class of substances not amenable to this modification is wheat products. In the yeast-fermentation method, a partial solution of the difficulties arising from the sulfite correction procedure is obtained through the use of excess hydrogen peroxide in removing residual sulfite. If a sufficient number of analyses are made (3 to 5), values obtained by this procedure usually agree satisfactorily with thiochrome results. In the assay of alkali-treated materials the fermentation method indicates the presence of several times as much thiamine as does the thiochrome method.

FOR comparative purposes the thiamine content of various types of samples has been determined by yeast-growth, yeast-fermentation, and thiochrome methods. Along with this comparative study, some work has been done on possible modifications of the methods involving yeast. Among the samples assayed were two (15 and 16, Table I) used for checking purposes by the American Association of Cereal Chemists and three samples (9, 11, and 13, Table I) used in collaborative studies sponsored jointly by the Research Corporation of New York and the American Association of Cereal Chemists. Included also were samples which had been subjected to heat treatment in alkaline solution; the thiamine content of these, in which deliberate destruction of the vitamin had been effected, was estimated by the methods under consideration.

The thiochrome and yeast-fermentation methods have been subjected to comparative study and standardization by Frey and Hennessy (7) and collaborators. The mean values obtained by the two methods for cereal samples and dry yeast agree remarkably well and are in agreement with mean values obtained using animal methods (rat-growth and rat-curative); but the range of deviation of individual values from a given mean is considerable, in the fermentation method being as high as 59%, in the chemical one as high as 122%. These studies were made on cereal products with one exception, and values for each sample obtained by a single method vary considerably.

Cheldelin and Williams (2) report that thiamine values for food samples determined by the yeast-growth method of Williams, McMahan, and Eakin (20) are in good agreement in most cases with the values obtained by the thiochrome method as reported by Lane, Johnson, and Williams (13), Nordgren and Andrews (15), and Conner and Straub (3); these comparisons, however, were not made on the same sample preparations. Only in the case of foods which have been subjected to processing involving heat treatment did Cheldelin and Williams observe the yeast-growth method to show striking disagreement (too high values).

No previous comparisons have been made using the yeast-growth and yeast-fermentation methods.

In a comparative study such as this, it is desirable to prepare extracts of samples which are suitable for assay by each method employed. Since thiamine often occurs in yeast and animal tissues as cocarboxylase, and since the pyrophosphoric ester is inactive in the yeast growth test (20) and thiochrome pyrophosphate is not extractable with isobutanol (12), a hydrolyzing agent must be used to convert cocarboxylase to thiamine if the yeast-growth or thiochrome methods are to be used.

Lohmann and Schuster (14) have shown that the above conversion can be accomplished enzymatically with suitable enzyme preparations. Pyke (17) and Dawson and Martin (4) have used digestion with pepsin followed by digestion with takadiastase, and Harris and Wang (8) have used incubation with takadiastase and papain following a preliminary heating in the presence of acid. Cheldelin *et al.* (1) used digestion with takadiastase and papain, and in this study their procedure was adopted; the phosphatase preparation used is sold under the trade name Clarase.

### ASSAY METHODS

**YEAST GROWTH.** The method of Williams, McMahan, and Eakin (20), which is based on the stimulatory effect of thiamine on the growth of *Saccharomyces cerevisiae*, Old Process strain, was used. Yeast growth was measured turbidimetrically.

Though the basic procedure of the growth method was retained throughout this work, the preparation and treatment of extracts to be assayed were varied. Thiamine content of the following types of extracts was estimated: (a) extracts of enzyme-digested samples, (b) extracts of alkali-digested samples, and (c) eluates of extracts (a) and (b) prepared by adsorption of sample aliquots on Decalso followed by elution with acidified potassium chloride.

**YEAST FERMENTATION.** The procedure recommended by Schultz, Atkin, and Frey (18) was followed in determining the thiamine content of the following types of sample extracts: (a) extracts of enzyme-digested samples, and (b) extracts of alkali-digested samples.

A commercial fermentometer was used.

In some cases this method was modified to the extent that, instead of using sulfite treatment as a means of correcting for non-thiamine activity, preliminary adsorption and elution using Decalso were performed before the fermentation test was applied.

**THIOCHROME.** The procedure of Hennessy (9) was followed, especial care being taken to standardize the timing of all operations beginning with the oxidation of the sample. The thiamine content of eluates of the following types of extracts was estimated: (a) extracts of enzyme-digested samples, and (b) extracts of alkali-digested samples.

Fluorescence was determined with a Pfaltz-Bauer fluorophotometer.

### PREPARATION OF SAMPLE EXTRACTS AND ELUATES

**ENZYME-DIGESTED SAMPLES (1a).** In order to obtain certain materials to be assayed in a finely divided condition, they were homogenized in a Waring Blendor. Two per cent Clarase and 2% papain were added to each sample, together with 0.5% sodium acetate-acetic acid buffer (pH 4.5), and after the addition of 0.5 ml. of benzene the mixture was incubated for 24 hours. At the end of the incubation period the samples were steamed 30 minutes, made to volume, filtered, steamed 10 minutes for sterilization, and then stored in the refrigerator until assayed. The amount of buffer used in preparing the respective extracts depended on the approximate thiamine content of the sample, since Hennessy (9) has found preferred volumes from which adsorption should be effected, as well as preferred amounts of thiamine to be adsorbed per column of Decalso.



This procedure was varied somewhat for samples of high starch content; for these, in addition to an extract prepared in the manner above, an extract was prepared by diluting the incubated mixture to its final volume and filtering prior to the 30-minute steaming period. The extract was refiltered if solid material separated out on steaming. This type of extraction is designated as 1a' in Table I.

**ALKALI-DIGESTED SAMPLES (2a).** The samples listed in Table II were put in solution, the pH was adjusted to 9 by addition of sodium hydroxide, and the solution autoclaved 1 hour at 7 kg. (15 pounds) pressure. After cooling, the pH of each solution was adjusted to 4.5 by the addition of sulfuric acid and the volume brought to its final value.

**ELUATES (1b, 1b', 2b).** An aliquot of each of the sample extracts containing 0.5 to 10 micrograms of thiamine was adsorbed on a column of Decalso and eluted with acidified potassium chloride, in accordance with the method described by Hennessy (9). The treatment of the zeolite prior to adsorption and the entire base-exchange procedure used were those recommended by Hennessy.

DISCUSSION OF METHODS

**YEAST GROWTH.** Williams and co-workers (16, 21, 23, 24) have used the yeast-growth method in investigations of small amounts of tissues, in which all other methods were of necessity excluded because of the relatively large samples required. It was found to be highly sensitive and to give reproducible and seemingly consistent results of the right order of magnitude; recovery tests indicated that it was sufficiently specific to be of value when applied to fresh tissue extracts. It requires inexpensive equipment and many tests per day can be run by one individual. However, the finding of Cheldelin and Williams (2) that yeast-growth values for materials which have been heated during processing are much higher than corresponding thiochrome values has made it evident that the yeast-growth method cannot be applied in its original form to certain types of materials.

A modification has proved useful in overcoming this discrepancy. The thiamine in sample extracts was separated from other materials known to stimulate yeast growth by taking advantage of the selective adsorption of Decalso for the vitamin,

and the contents of the eluates from adsorption were measured. Neither 5-(2-hydroxyethyl)-4-methylthiazole, which was found in this investigation to be 68% as active as thiamine on an equimolecular basis, nor 4-amino-5-ethoxymethyl-2-methylpyrimidine which is 14% active, is adsorbed on Decalso from solutions in sodium acetate buffer (pH 4.5) in the routine adsorption procedure. [Deutsch (5) reports that "more than 95% of the pyrimidine is also removed by zeolite"; the pyrimidine referred to is presumably "(III) the pyrimidine portion, 4-amino-2-methyl-5-ethoxymethylpyrimidine". He fails to indicate, however, whether or not the adsorbent used was activated Decalso and at what pH adsorption was effected.] Indirect evidence points to an analogous adsorption behavior of the 5-hydroxymethylpyrimidine derivative which is formed by the hydrolytic cleavage of thiamine, since eluates from the Decalso adsorption of alkali-treated samples (Table II) measured in the yeast-growth method proved to have only a small percentage of the activity determined for the corresponding whole extracts.

**YEAST FERMENTATION.** In preliminary investigations made in connection with work done at this institution (22) in determining the thiamine content of food samples, considerable difficulty has been encountered in obtaining satisfactory replicate values for the sulfite-treated samples. This difficulty was removed in part by the use of excess hydrogen peroxide in destroying residual sodium sulfite, a precautionary measure recommended by Josephson and Harris (11) for the microfermentation method. But for some samples this precaution did not alleviate the difficulty. It has been reported recently that the thiazole and pyrimidine sulfonic acid resulting from the sulfite cleavage of thiamine are slightly stimulatory in the microfermentation method (5), and it is not unlikely that they are similarly active in the macromethod.

A modification involving adsorption was attempted in order to eliminate the sulfite treatment of samples, but it failed to eliminate entirely the interfering substances. It was established that filtrates with combined washings from Decalso adsorption of

Table I. Comparison of Values from Thiochrome, Yeast-Growth, and Yeast-Fermentation Determinations

Sample	I Thiochrome (1b, 1b')	II Yeast Growth (1b, 1b')	III Yeast Growth (1a, 1a')	IV Yeast Fermentation (1a, 1a')	V Yeast Fermentation (1b, 1b')	Deviation					
						II from I	II from IV	III from I	III from IV	IV from I	V from I
						%	%	%	%	%	%
Plant and animal tissues											
Micrograms per gram											
1 Peanuts, fresh (1a)	7.1	7.0	14.7	7.1	7.2	- 1.4	- 1.4	+ 107	+ 107	0	...
2 Oatmeal (1a)	6.8	7.9	8.1	8.0	7.2	+ 15.6	+ 1.2	+ 18	+ 1.2	+ 16.8	+ 5.9
3 Oatmeal (1a')	7.6	9.5	10	8.4	6.9	+ 25	+ 13	+ 32	+ 19	+ 10.5	+ 9.2
4 Beef muscle (1a)	0.59	0.63	1.3	0.94	0.76	+ 6.8	- 33	+ 114	+ 34	+ 59.4	+ 29
5 Potatoes, white (1a)	1.13	1.18	1.82	1.41	1.34	+ 4.4	- 16	+ 61	+ 29	+ 25	+ 19
6 Potatoes, white (1a')	1.11	1	1.65	1.21	1.22	- 9.9	- 8.2	+ 49	+ 36	+ 9	+ 9.9
7 Potatoes, white (dehydrated) (1a)	2.97	5	4.25	3.3	..	+ 68	+ 51	+ 43	+ 29	+ 11	...
8 Green peas (dehydrated) (1a)	5.77	6.9	8.5	7.5	..	+ 20	- 8	+ 47	+ 13	+ 30	...
9 Dry yeast (1a) <sup>a</sup>	598	658	735	654	625	+ 10	+ 0.6	+ 23	+ 13	+ 9.4	- 5.4
10 Wheat germ (1a)	18.4	24.5	20.6	16.7	..	+ 33	+ 47	+ 12	+ 23	- 9.2	...
11 Whole wheat (1a) <sup>a</sup>	4	6.65	..	5.2	5.1	+ 66	+ 28	...	...	+ 29	+ 27
12 Whole wheat (1a')	4.2	5.2	5.2	4.3	5.0	+ 24	+ 21	+ 24	+ 21	+ 24	+ 19
13 White flour (1a) <sup>a</sup>	0.69	1.24	..	0.81	1.34	+ 79.5	+ 53	...	...	+ 17	+ 94
14 White flour (1a')	0.68	0.93	0.98	0.57	1.01	+ 37	+ 63	+ 44	+ 72	- 16	+ 49
15 Cereal product (1a) <sup>b</sup>	24.9	30.3	31.9	25.9	26.8	+ 22	+ 19	+ 28	+ 23	+ 4	+ 7.6
16 Rice product (1a) <sup>b</sup>	2.8	3.4	4.6	3	4.9	+ 21	+ 13	+ 64	+ 53	+ 7	+ 75
Processed materials <sup>c</sup>											
17 Peanuts, roasted (1a)	2.8	3.3	15.3	2.8	..	+ 17.8	+ 17.8	+ 446	+ 446	0	...
18 Yeast extract (1a)	17.1	19.4	63.6	23.7	..	+ 13.4	- 18.2	+ 272	+ 168	+ 38.6	...
19 Rice bran concentrate (1a)	50.2	51.2	170	75.8	..	+ 2	- 32.4	+ 240	+ 124	+ 51	...
20 Whole wheat bread (1a)	1.2	2.2	2.4	1.03	..	+ 83	+ 114	+ 100	+ 133	- 13	...
Urine											
Micrograms per ml.											
21 Sample 1 (1a)	0.061	0.081	..	0.23	0.5	+ 33	- 65	...	...	+ 277	+ 719
22 Sample 2 (1a)	0.15	0.18	1.23	0.54	..	+ 20	- 67	+ 720	+ 128	+ 260	..

<sup>a</sup> Received from R. R. Williams. These samples, designated as Nos. 6, 1, and 3 by the Research Corporation Committee, have respective thiamine contents of 702, 5.1, and 0.92 micrograms per gram. Indicated mean values were derived from results of nineteen collaborators who used thiochrome method (7). Many individual values deviated widely from the mean.

<sup>b</sup> Obtained from J. S. Andrews, of General Mills, Inc. Thiamine content, 25 and 3.0 micrograms per gram, respectively.

<sup>c</sup> Subjected to more or less cooking, may contain thiamine fragments.



samples contain considerable quantities of material stimulative to fermentation, and further that 4-amino-5-ethoxymethyl-2-methylpyrimidine, and probably the corresponding 5-hydroxymethylpyrimidine derivative as well, each of which stimulates fermentation (18, 19), are not adsorbed on Decalso under the conditions employed. Hence it appeared that eluates from Decalso adsorption might serve as suitable test materials. The salt concentration in the eluate depresses fermentation somewhat, so that it was necessary to add to each standard bottle the amount of potassium chloride in the aliquot of eluate being tested. In no case was more than 5 ml. of an eluate used.

In checking the fermentometer it was found that some of the bottles, regardless of placement in the shaker, permitted settling of the yeast during the 3-hour shaking period, and as a consequence smaller volumes of carbon dioxide were evolved than from those bottles in which no sedimentation occurred. This source of error was easily eliminated by replacing the imperfect bottles which had irregular seals between the walls and bottoms.

**THIOCHROME.** In this method based on the oxidative conversion of thiamine to thiochrome, a possible source of error arises from the fact that thiamine may be incompletely adsorbed on the zeolite. Egaña and Meiklejohn (6) have found by means of recovery experiments that urine samples which contain blood or bile, as well as those from individuals whose thiamine intake is low, contain some material which prevents complete adsorption of the vitamin on Decalso, while normal urine appears free from this inhibitory material. Such inhibitory substances may be of more general occurrence than is appreciated, in which case the effects would be particularly apparent in the case of extracts containing a low concentration of thiamine. That urine contains an unidentified material which interferes with the adsorption of pantothenic acid on charcoal was found by Hogg (10).

The presence of interfering fluorescent materials in the eluates can also serve as a source of error, especially if such materials are labile to oxidation, so that their contribution to fluorescence in the oxidized sample differs from that in the unoxidized blank.

Values obtained by the chemical method have been chosen as reference values for this comparative study.

Table II. Effect of Alkali Treatment on Thiamine Content

Samples	Thiochrome (2b)	Yeast Growth (2b)	Yeast Growth (2a)	Yeast Fermentation (2a)
		<i>Micrograms per gram</i>		
Yeast extract (2a)	0.02	1	65.2	5.8
Yeast extract (2a)	0.33	2.57	46	3.9
Rice bran concentrate (2a)	3.6	9.3	187	18.7
Thiamine, crystalline (2a)	0	4 <sup>a</sup>	4.5 <sup>a</sup>	13 <sup>a</sup>

<sup>a</sup> Values in terms of % thiamine activity intact after alkali treatment.

#### DISCUSSION OF RESULTS

The comparative results obtained for enzyme-digested samples, together with the percentage deviation of values from corresponding thiochrome values, are listed in Table I. In the case of values from yeast-growth determinations, percentage deviations from yeast-fermentation results are also indicated. In Table II, values for the thiamine content of the alkali-treated samples are presented.

Of the samples assayed in this study which have been used previously in two sets of collaborative determinations, Nos. 15 and 16 were found to give thiochrome and fermentation values in close agreement with reported contents, but values lower than the reported means were obtained for Nos. 9, 11, and 13. Since the authors' values for the last three were obtained at least a year and a half later, it is not unlikely that in these samples some loss of thiamine occurred during storage.

**COMPARISON OF VALUES FROM YEAST-GROWTH AND THIOCHROME DETERMINATIONS.** Without exception, values obtained for whole extracts (1a, 1a') by the yeast-growth method are higher

than corresponding values from thiochrome determinations. In the case of heat-processed materials and urine, the deviation are of far greater magnitude than for the unprocessed materials assayed. This is probably due to the presence of active fragments of the thiamine molecule in these samples.

For most samples, values for eluates (1b, 1b') are significantly lower than those for the corresponding whole extracts in the yeast-growth determination, and are in better agreement with values from the chemical method. Fifteen of the 22 eluates listed in Table I gave values within  $\pm 25\%$  of the thiochrome values, of them being within  $\pm 10\%$ . For wheat products, use of the adsorption technique accomplishes no improved agreement of values from yeast-growth assay with those from thiochrome determinations. Evidently wheat products contain some growth-promoting substance other than thiamine which is not separated from the vitamin by the base-exchange procedure.

**COMPARISON OF VALUES FROM YEAST FERMENTATION AND THIOCHROME DETERMINATIONS.** Values listed in Table I obtained by thiochrome and yeast-fermentation assay are in fair agreement, 15 of the 22 fermentation values agreeing within  $\pm 25\%$  with thiochrome figures. In the case of rice bran concentrate, beef muscle, and urine, fermentation values are more than 50% higher than thiochrome values. For rice bran concentrate, the nonthiamine material active in the fermentation test (determined by sulfite treatment) amounts to approximately 50% of the total stimulatory material present; for urine, it represents approximately 70% of the total. These correction terms are certainly high, but such corrections do not of themselves invalidate results because in other samples assayed—viz., roasted peanuts, yeast extract, whole wheat bread, and white flour—the nonthiamine active material amounts to 50% or more of the total stimulatory material present, yet agreeing values are obtained by the two methods. It may be that the rice bran concentrate, beef muscle, and urine samples contain some material which interferes with the adsorption of thiamine on Decalso, so that values from the fluorometric method are lower than the actual thiamine content; that urine may contain such material has been pointed out by other workers (6). An alternate interpretation is that these samples contain some active material other than thiamine which is inactivated by sulfite treatment, so that it appears as thiamine in the fermentation assay.

For those eluates assayed in the yeast-fermentation method, values were obtained which for the most part agree as well or better with thiochrome values than do the corresponding values obtained using sulfite treatment. For urine, white flour, and the rice product this was not the case; for these, values for the eluates were considerably higher. Since the assay of eluates does not give consistently improved values, it cannot be recommended as an adequate substitute for the usual procedure involving sulfite correction.

**COMPARISON OF VALUES FROM YEAST GROWTH AND YEAST FERMENTATION DETERMINATIONS.** Yeast-growth values for whole extracts (1a, 1a') of unprocessed materials are from 1 to 100% higher than corresponding fermentation values; values for processed materials are entirely out of line, as observed in the previous comparison. Values for eluates (1b, 1b') assayed by yeast growth are in better agreement with fermentation results and for most samples the agreement is fairly good; 12 of the 22 eluate values in Table I agree with the corresponding fermentation values within  $\pm 25\%$ . Dehydrated potatoes, white flour, wheat germ, and whole wheat bread gave discordant results by the two methods.

**COMPARISON OF VALUES FOR ALKALI-TREATED SAMPLES.** The results listed in Table II show that pure thiamine subjected to heat treatment at an alkaline pH is, according to thiochrome measurement, completely destroyed. In the yeast-growth method it retains 4 to 4.5% of its activity, and in the yeast-fermentation, 13%.

For alkali-treated samples tested, thiochrome values are far



over than are corresponding values from either of the yeast methods. Eluate yeast-growth values are in better agreement with thiochrome values than are any others, but they too are significantly higher.

In each extract there appears to be some material which is (1) relatively stable to alkali and heat treatment, (2) adsorbed on Decalco and eluted with acidified potassium chloride, (3) labile to sulfite treatment, and (4) stimulatory to yeast growth and fermentation.

#### ACKNOWLEDGMENT

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## Device for Rapid Closing of Weighing Bottles

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WHEN large numbers of vacuum-oven moisture determinations are made, it is convenient to use a device designed to facilitate closing a number of weighing bottles at the same time. Closing each bottle by hand is not only tedious but slower, and this delay between opening the oven and closing the bottles may give some samples a chance to absorb moisture from the atmosphere.

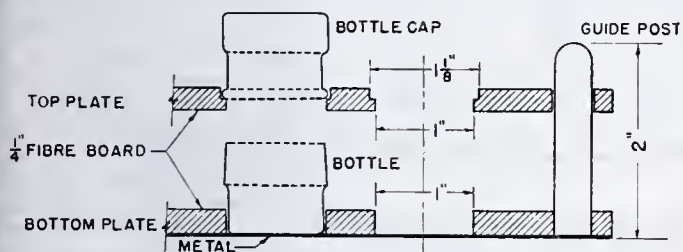


Figure 1. Type A. Cross Section Showing Openings for Bottles and Guide Post

Two devices, fundamentally alike but adapted to different forms of bottle closure, have been used in this laboratory. Each consists of two plates. The top plate supports the lids or stoppers and serves both to open and to close the bottles; the bottom plate, similar in both types, keeps the bottles in fixed positions. Both plates are placed in the oven where they rest on the brackets that ordinarily support the shelves.

Plates of type A (Figure 1) were designed to fit a vacuum oven in the form of a cylinder 22.5 cm. (9 inches) in diameter by 45 cm. (18 inches) long, and the pair accommodates 40 Parr weighing bottles, 25 mm. wide and 20 mm. high, having outside-fitting ground-glass lids. The bottom plate consists of a continuous metal sheet, 0.16 cm. (1/16 inch) thick, riveted to thicker 0.6-cm., 0.25-inch nonmetallic sheet material (such as fibre-board) having round holes, uniformly arranged in parallel rows, to fit the bottoms of the bottles. Metal is used for the continuous base to ensure good heat conductivity to the bottles. The top plate, which holds the lids, is made of nonmetallic sheet material and has holes corresponding to those in the bottom plate and large enough to let the uncovered weighing bottles go through. These holes have a slightly larger diameter from the upper surface to about halfway through the plate, in order to

hold the lids in place. One end of the top plate has a hole corresponding in position and size to a guide post at one end of the bottom plate.

To load this device, the top plate is placed on the bottom one, the closed bottles are placed in the holes, their lids are loosened, and the top plate is slowly raised. The bottoms of the bottles will remain on the lower plate, while the lids will be lifted with the top plate. After the drying period, the bottles are closed by moving the upper plate, loaded with the caps, downward along the guide post. The closed bottles may then be lifted out of the holes.

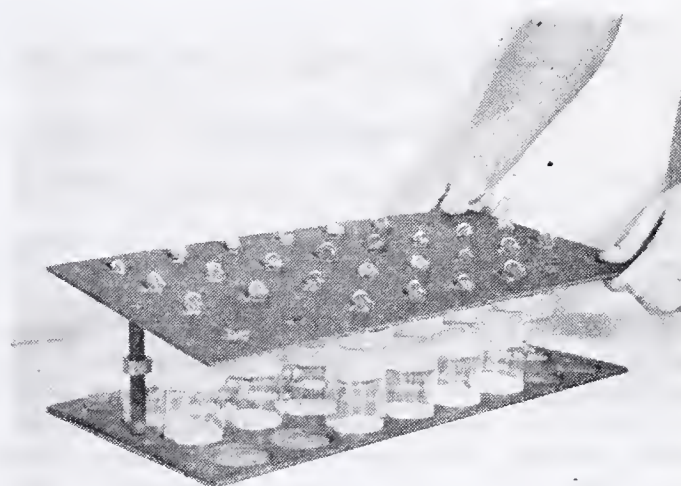


Figure 2. Type B. Stoppers Being Lowered onto Bottles

A similar device, type B (Figure 2), accommodates 32 bottles, 40 mm. wide by 50 mm. high, having inside-fitting ground-glass stoppers. The top plate has slits and grooves at right angles to each other. All the slits are parallel. The bottles are placed in the bottom plate with the flattened tops of the stoppers parallel to the direction of the slits in the top plate, which is lowered until it rests on the weighing bottles; the tops are turned through a 90° angle to fit into the grooves; and the top plate is slowly lifted, removing the stoppers which are suspended from it. After drying, the stoppers may be lowered onto the bottles, the tops turned in the direction of the slits, and the top plate lifted off.

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# Substituted 1,10-Phenanthroline Ferrous Complex Oxidation-Reduction Indicators

## Potential Determinations as a Function of Acid Concentration

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This paper deals with determination of the variation in the potential at which the phenanthroline ion is oxidized from the ferrous to the ferric form as a function of acidity. The data include those for the substituted complex ions in which the 5- or 6-position hydrogen is replaced by methyl, nitro, chloro, or bromo radicals, and for the complex ions in which the 5- and 6-position hydrogens have been replaced by both the methyl and nitro groups.

THE first application of the phenanthroline complex ion as an indicator in oxidation-reduction reactions was described by Walden, Hammett, and Chapman (8). The nitro substitution product was first studied by Hammett, Walden, and Edmonds (1), and its first practical application was in the determination of oxalic acid, described by Smith and Getz (6). The synthesis of the materials is described by Smith and Getz (5) and Richter and Smith (4). The spectrophotometric properties of these products were studied by Moss, Mellon, and Smith (3).

Titration using 0.1 *N* potassium dichromate dissolved in sulfuric acid (4 gram molecules of sulfuric acid per liter)

Frequently employed reference point potentials of various systems as used at different acid concentrations are given in Table II.

### GENERAL PROCEDURE FOR DETERMINATION OF FORMAL ELECTRODE POTENTIALS

For some of the phenanthroline ions the potential of the higher reference system is not sufficient to oxidize the ferrous to the ferric ion complex. To use a perchloric acid solution of perchloratoceric acid because of its higher potential in some cases was unsatisfactory because of the formation of insoluble ferrous perchlorate complex phenanthroline ions. In other cases equal insoluble sulfuric acid complex ions resulted at the higher acidities.

The ferric complex phenanthroline ions (with the exception of oxidized ferroin and methyl-ferroin) were not stable for more than a short interval, especially at higher acid concentrations.

In these cases solutions of 0.01 or 0.025 *N* sulfatoceric ion solutions containing 1 to 8 gram molecules of sulfuric acid per liter were prepared by dissolving pure ammonium nitratocerate in concentrated sulfuric acid followed by gradual dilution to the proper amount. The substituted ferroin indicator solutions of 0.01 or 0.025 *M* concentration were prepared by solution of the proper weight of indicator base (Table I) in 0.01 or 0.025 *M* ferrous ammonium sulfate solution. Measured portions of the cerate solution (25.00 ml.) were placed in 400-ml. beakers and an equal volume of sulfuric acid of twice the strength finally required was added. Dilution was

Table I. Physical Constants of Phenanthrolines and Their Substitution Products

Compound	M. P. (Anhydrous) ° C.	Common Name of Ferrous Sulfate Complex	Mol. Wt.	Amount Required for 1000 Ml. of 0.01 <i>M</i> Fe <sup>++</sup> Complex Grams	Formal Oxidation, E.M.F., Volts <sup>a</sup>
5-Nitro-1,10-phenanthroline	202	Nitro-ferroin	225.20	6.7560	1.25
5-Nitro-6-methyl-1,10-phenanthroline	269	Nitromethyl-ferroin	239.23	7.1768	1.22
5-Bromo-1,10-phenanthroline monohydrate	119	Bromo-ferroin	277.11	8.3134	1.12
5-Chloro-1,10-phenanthroline monohydrate	123	Chloro-ferroin	232.66	6.9800	1.12
1,10-Phenanthroline monohydrate	117	Ferroin	198.22	5.9464	1.06
5-Methyl-1,10-phenanthroline monohydrate	114	Methyl-ferroin	212.24	6.3673	1.02

<sup>a</sup> Formal potential, when oxidized and reduced forms are equal, in sulfuric acid solutions of one molecular weight per liter (without reference to their possible incomplete ionization, hydrolysis, formation of complexes, etc.) and at 25° C. (7).

### EXPERIMENTAL WORK

Pertinent data concerning the materials of this discussion are contained in Table I.

A procedure similar to that described by Walden, Hammett, and Chapman (8) was employed when both the ferrous and ferric complex phenanthroline ions were found to be stable in the various strengths of acid studied. This was true in the case of 1,10-phenanthroline and 5-methyl-1,10-phenanthroline. The simultaneous titration of a mixture of ferrous sulfate and ferrous phenanthroline ions was carried out, using either a solution of sulfatoceric acid or potassium dichromate in solutions of sulfuric acid of concentration equal to that of the solutions of the ions being titrated. The range of acidity employed was from 1 to 8 moles per liter. The ceric-cerous and ferrous and ferric potentials were separately determined under the same conditions and the values of these two reference points reconfirmed.

A typical sample titration graph is shown in Figure 1. Titration conditions used were:

10 ml. of an approximately 0.1 molar ferrous sulfate solution in sulfuric acid (4 gram molecules of sulfuric acid per liter)

15 ml. of an approximately 0.1 molar solution of methyl-ferroin added to an equal volume of sulfuric acid (8 gram molecules of sulfuric acid per liter)

Dilution to 200 ml. by addition of sulfuric acid (4 gram molecules of sulfuric acid per liter)

made to 200 ml. with sulfuric acid of the same desired strength

A measured portion (50.00 ml.) of the ferroin or substituted ferroin was then added in one portion with vigorous stirring. The potential of the resulting solution was read at once, using saturated calomel electrode and a bright platinum electrode references. Any condition such as results from instability of the ferric phenanthroline ions was indicated by a gradual fall in potential which could be observed without difficulty. Such instability was more pronounced at higher acid concentrations and

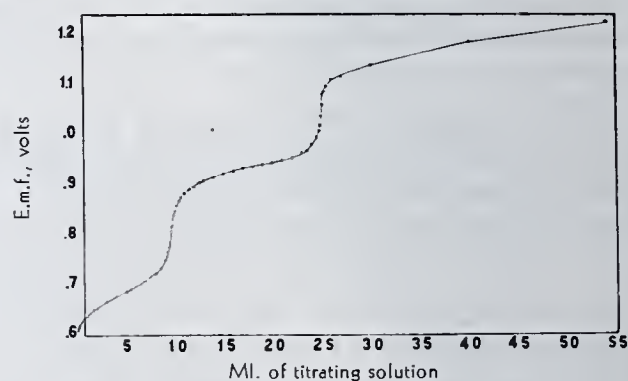


Figure 1. Simultaneous Titration of Ferrous and Phenanthroline Ferrous Ions

In 4 *M* sulfuric acid solution, using potassium dichromate in 4 *M* sulfuric acid throughout



**Table II. Formal Electrode Potentials of Various Systems in Sulfuric and Hydrochloric Acid Solutions<sup>a</sup>**

Potential Determined	Sulfuric Acid Concentrations (Moles per Liter)				
	1	2	4	6	8
E.M.F., Volts					
$\text{Fe}^{+++} \longleftrightarrow \text{Fe}^{++}$	0.68	0.68	0.68	0.68	0.68
$\text{Cr}_2\text{O}_7^{--} \longrightarrow 2\text{Cr}^{+++}$	...	1.11	1.15	1.30	1.35
$\text{Ce}(\text{SO}_4)_3^{--} \longrightarrow \text{Ce}^{+++}$	1.44	1.43	1.42	...	1.40
Potential Determined	Hydrochloric Acid Concentrations (Moles per Liter)				
	1	2	3	4	
E.M.F., Volts					
$\text{Fe}^{+++} \longleftrightarrow \text{Fe}^{++}$	0.69	0.68	0.67	0.66	
$\text{Cr}_2\text{O}_7^{--} \longrightarrow 2\text{Cr}^{+++}$	1.09	1.11	1.19	1.15	

<sup>a</sup> Determinations of present study are taken from Smith and Getz (6).

**Table III. Formal Oxidation Potential of Ferroin and Substituted Ferroin Indicators at Various Strengths of Sulfuric Acid**

Indicator	Sulfuric Acid Strength					
	0.5 M	1 M	2 M	3 M	4 M	8 M
Oxidation Potential, Volts						
nitro-ferroin	1.26	1.25	1.22	...	1.12	1.11
nitromethyl-ferroin	...	1.23	...	...	...	...
romo-ferroin	1.13	...	...	...	...	...
chloro-ferroin	...	1.11	1.10	...	1.04	0.97
ferroin	...	1.06	1.03	1.00	0.96	0.89
ethyl-ferroin	...	1.02	1.00	0.96	0.93	0.86
2'-Dipyridyl-ferroin	...	0.97	...	...	0.92	...

th ferric phenanthroline ions of highest electrode potential. ie systems showed no appreciable change in potential during e time required for reading the potential of the first mixing.

The data obtained are found in Table III. By determination potentials in many cases by both procedures, the values were own to be reliable within 0.02 volt. The values obtained by her procedure duplicated those of Walden, Hammett, and their -workers as corrected by Hume and Kolthoff (2). As previ- sly assumed (2), the oxidation potential of the bipyridinium ous complex ion is not so high as that of ferroin.

In using the data of Tables II and III as a guide to titrations ploying visual equivalence point determinations rather than tentimetric observations, it must be kept in mind that the

color change from red in reduced solutions to faint blue in oxidized solutions requires approximately 90% oxidation of the indicator ion before the red hue is eliminated. The oxidation potential is thus effectively approximately 60 millivolts higher than the values give in Table III. The values given in Table III are claimed to be valid to within  $\pm 20$  millivolts and in most cases better.

### SUMMARY

Formal oxidation potentials of the ferric-ferrous and the dichromate-chromic systems have been determined in 1 to 8 M sulfuric and hydrochloric acid solutions. The use of such data in the selection of the proper indicator systems for determination of reaction and points is suggested.

A general procedure for use in determination of the formal electrode potentials of reversible oxidation-reduction indicators of the ferroin and substituted ferroin group is described.

The oxidation potential of the phenanthroline ion and nitro, bromo, chloro, methyl, and nitromethyl phenanthroline ions is given in various sulfuric acid strengths from 1 to 8 M.

For the system of indicators studied the range of oxidation potentials found varies from 0.7 to 1.26 volts, with all gradations between represented.

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ABSTRACT of a portion of a thesis presented in partial fulfillment of the requirements for the Ph.D. degree in the Graduate School, University of Illinois.

## Use of Synthetic Detergents in the Van Slyke Determination of Oxygen Capacity

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MODIFICATIONS of the original Van Slyke procedure (5, 7) for the determination of blood oxygen capacity have been concerned chiefly with mechanical and manipulative improvements (1, 2, 3, 6, 8, 9). It occurred to the authors to test several synthetic detergents, of different types, as possible substitutes for the saponin prescribed by Van Slyke as the hemolytic agent.

The results below indicate that several common detergents may conveniently be used in place of the less readily available, more expensive, and mildly irritating saponin. Sendroy's procedure has been used in these determinations on rabbit and horse blood. It is reasonable to assume that the modified method can be extended to the blood of other species.

A saturated solution of each of the detergents, with the exception of the RO-C, was prepared in a freshly made potassium ferri-ferrocyanide solution containing 23 grams per 100 cc. The saturated solutions were prepared by adding one volume of potassium ferri-ferrocyanide of twice the desired concentration to an equal volume of detergent solution containing 16 grams per 100 cc and filtering. The source of each of the detergents used can be ascertained by reference to the 1943 list (4). In the case of the RO-C (a cationic detergent of the alkyl dimethylbenzyl

**Table I. Oxygen Capacity Determinations on Fresh Oxalated Rabbit Blood Diluted with 1% NaCl Solution**

Detergent	Type	Volume % O <sub>2</sub>
Saponin	Natural polycyclic glucoside, Merck	11.99
Duponol WA	Long-chain alcohol sulfate	12.06
Aerosol O.T.	Sodium dioctyl sulfosuccinate	11.72

ammonium chloride type, Winthrop Chemical Company), 8 grams per 100 cc. were used, an amount equal to that of the saponin prescribed by Van Slyke. The RO-C reacted slowly with potassium ferri-ferrocyanide and is not considered suitable for use with it as the oxidizing agent.

The results in Table I suggest that Duponol WA may be readily employed in place of saponin, but that the use of Aerosol O.T. yields slightly low values. A favorable check in this analysis is  $\pm 0.2$  volume % (8). All determinations, including blanks, were carried out in duplicate.

Table II indicates that each of the three detergents tested will give satisfactory results on fresh oxalated rabbit blood diluted with 1% sodium chloride. The use of Nacconol FSNO, an alkyl aryl sulfonate type, led to similar values. A freshly prepared



Table II. Oxygen Capacity Determinations

Detergent	Type	Volume % O <sub>2</sub>
Saponin	Merck product	10.40
Duponol W-20	Long-chain alcohol sulfate	10.39
Aerosol O.S.	Isopropyl naphthalene sodium sulfate	10.49
Arctic Syntex M.	Sulfate of glycerol monolaurate	10.52

and used RO-C-potassium ferricyanide combination led to slightly low results.

Additional experiments also indicated that either potassium dichromate or iodine in 10% potassium iodide may be substituted in equimolecular amounts for potassium ferricyanide and used with saponin. This aspect of the problem was not pursued further.

Accordingly, in view of the experiments described in this report, it is suggested that synthetic detergents of the long-chain

alcohol sulfate type, the alkyl aryl sulfonate type, or the mono-glyceride sulfate type be used as hemolytic agents in the determination of blood oxygen capacity with potassium ferricyanide as the oxidizing agent. It is, of course, possible that other readily available detergents may be equally effective.

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FROM the senior thesis of M. A. Swerdlow, February, 1944.

## Quantitative Method for Determination of Maltose in the Presence of Glucose

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**A**N ACCURATE and rapid method is needed for analysis of mixtures of sugars, and especially for mixtures of maltose and glucose, but this method cannot be applied as outlined below to mixtures of these two sugars and other carbohydrates, such as "malt sirup" and "corn sirup".

Two methods cited by Browne and Zerban (1) are representative of the usual procedures that have been advocated: that of Morris, which combines copper reduction, polarization, and selective fermentation, and the shorter one of Steinhoff, which makes use of two copper solutions—i.e., a Soxhlet and a modified Barfoed. A more recent method is that of Schultz, Fisher, Atkin, and Frey (3), which is based on three fermentations, in which the evolved gas volumes represent the sugars acted upon, and the maltose and "β-amylase attackable substances" are computed by difference. This method is similar to that developed by the author at about the same time (2) for the "maltose fraction" in flour. Aside from the question of accuracy, these methods are involved and cumbersome.

Tomoda and Taguchi (4) have reported a polarimetric procedure for analysis of mixtures of glucose and maltose and of glucose and fructose similar to the one described herein but differing in detail. Their method has been condemned, apparently on the basis of misquotation of their statements regarding the accuracy of their maltose determinations. However, they claim that in four determinations of maltose in a maltose-glucose mixture the error was -1.10% in one and 0.0% in the other three.

The difference in the ability of various sugars to combine with bisulfites was noted by the author in the course of work on fermentations wherein bisulfites were present and this difference was made use of in the analyses of sugar mixtures for maltose. Although in the work of Tomoda and Taguchi the same principle was employed, it is believed desirable, because of the simplicity and accuracy obtainable, to describe a somewhat different method of application of this principle.

This polarimetric method is based on the fact that the optical rotation of glucose may be reduced to zero by addition of a sufficient quantity of soluble bisulfite, but the rotation of maltose and dextrans is affected only very slightly. Incidentally, the rotation of lactose and other reducing sugars is also lowered by the presence of bisulfites and the rotation of the sugar alcohols is unaffected. The speed and accuracy of this method are comparable with those of polarimetric determinations in general, but the sensitiveness is somewhat less.

The method of evaluation is based on Biot's additive rule of optical rotations—namely,  $[\alpha]_x = X[\alpha]_1 + (1 - x)[\alpha]_2$  when

$[\alpha]_x$  is the specific rotation of the mixture,  $[\alpha]_1$  and  $[\alpha]_2$  are the specific rotations of the individual components, and  $x$  is the fraction of one of them. Browne and Zerban (1) point out that the specific rotations used must take into account the solvent concentration. Since the concentration of the total sugars is constant, that of the water is approximately so, and an empirical relationship is adequate for this method, using observed values rather than specific rotations.

Table I. Optical Rotation of Maltose (Hydrate)-Dextrose (Anhydrous) Mixtures and Corresponding Percentages of Maltose

(In 30% bisulfite solution at 20° C. in 200-mm. tube)							
Maltose, %	0	20	40	50	60	80	100
Dextrose, %	100	80	60	50	40	20	0
° S. <sup>a</sup>	0	11.3	22.9	28.8	34.3	46.0	57.8
Maltose (calcd.), %	0	19.72	39.96	50.26	59.85	80.27	100.8

<sup>a</sup> ° S., degrees on International Sugar Scale.

## METHOD

The first requirement in the use of this method is a set of standard values for the optical rotation of maltose and glucose and mixtures of known proportions of these sugars in the presence of sodium bisulfite.

Because of the difficulty of dissolving relatively large quantities of bisulfite in sugar solutions of 10% or greater concentration, the writer prefers the following procedure in their preparation:

A series of seven solutions is prepared, each solution containing 10 grams of total sugar and not less than 75 ml. of water. The proportion of glucose to maltose should be 10 grams to 0, 8 to 2, 6 to 4, 5 to 5, 4 to 6, 2 to 8, and 0 to 10. To each of seven sugar flasks graduated to 110 ml. are added 30 grams of sodium metabisulfite or its equivalent of sodium bisulfite, and one of the sugar solutions is transferred to each. The flasks are shaken to dissolve the metabisulfite, cooled to 20° C., the contents made up to volume of 110 ml. with distilled water, mixed, and polarized at 20° C. The length of the polariscope tube need not be specified but should be the same for all determinations.

The observed rotations are then plotted against the percentage of maltose and will lie on practically a straight line defined by these points. The percentage of maltose present may be determined by referring the readings to the graph or by multiplying (° S.) by the tangent of the line, which in the present work was 1.745.



In Table I are given the optical rotations of solutions of maltose dextrose, *D*-glucose (anhydrous) and sodium metabisulfite by polarization in a 200-mm. tube at 20°, and the corresponding computed percentages of maltose.

The accuracy of the method is indicated by the values obtained. The maximum deviation from the actual values in terms of percent maltose was 0.28 and the minimum 0.04, with an average deviation of 0.20% for the five mixtures.

To determine the amount of maltose in a mixture of glucose and maltose, determine first the amount of total sugars by some accepted method. To each sugar flask used add 10 grams of the unknown mixture or the amount of its solution which contains 10 grams of total sugars. Dilute to approximately 75 ml. and pro-

ceed as described for the solutions of known sugar content. From the observed rotation the percentage of the total amount of sugar present as maltose can be calculated as described or may be determined with the use of the graph prepared from the data obtained upon the "known" solutions.

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## Estimation of Pyridine Content of Pyridine-Acetic Acid Mixture Used in Riboflavin Determination

J. H. LANNING AND C. A. ROSZMANN

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A PART of their procedure for the determination of riboflavin, Conner and Straub (1) used a pyridine-acetic acid mixture to elute the riboflavin from the Florisil adsorbent. After treatment with oxidizing agents, the fluorescence of the riboflavin in the eluate was determined. When a number of riboflavin determinations are made by their procedure, a considerable quantity of the used pyridine-acetic acid mixture is collected. After distillation, the pyridine together with water and acetic acid

Table I. Apparent Recovery of Pyridine from Mixtures

Pyridine in Original Solution	20.0 N Sodium Hydroxide		15.3 N Sodium Hydroxide	
	Water and pyridine mixture	Acetic acid, water, and pyridine mixture	Water and pyridine mixture	Acetic acid, water, and pyridine mixture
	Per Cent by Volume			
16.1	19.0	19.0	19.0	19.0
18.1	21.5	21.3	21.5	21.5
20.1	23.8	23.8	24.5	24.5
22.1	26.5	26.5	27.0	26.5
24.1	29.0	29.3	29.5	29.5

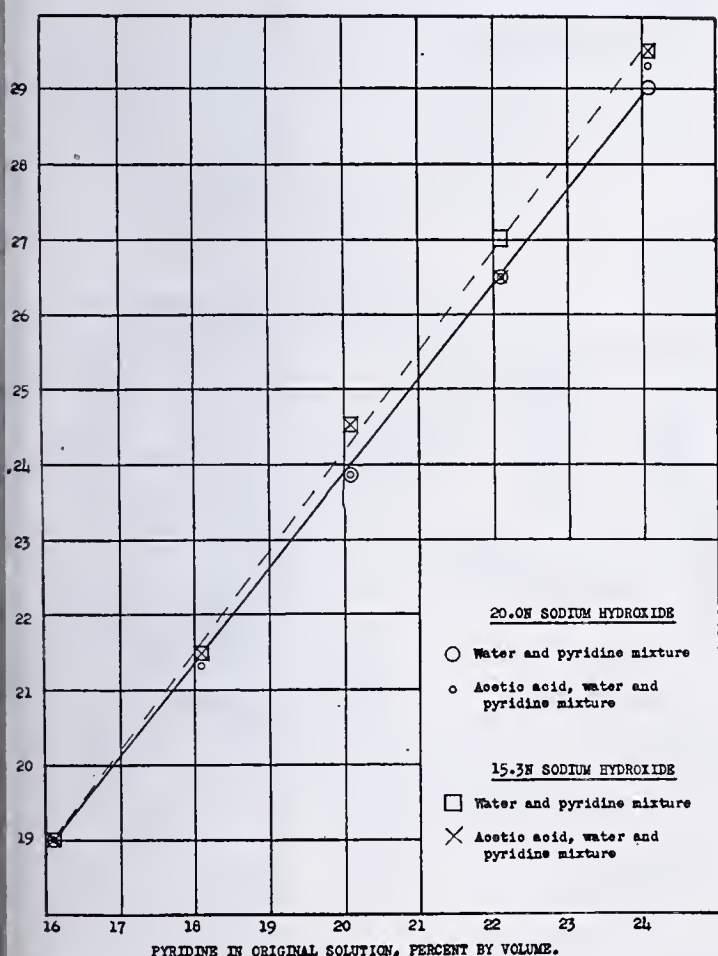


Figure 1.

may readily be recovered as a mixture, but unless some simple method is available for determining the concentration of these components, the mixture is worthless. The acetic acid may readily be determined by titration with 0.1 N sodium hydroxide, using phenolphthalein as indicator, and its concentration calculated in the customary manner, but a convenient standard method is not available for determination of the pyridine concentration. However, after some experimenting it was found that pyridine could be separated from the mixture by means of a strong solution of caustic soda. Using this principle, a method was devised for determining the approximate pyridine concentration of such a mixture.

**METHOD.** After distillation of the pyridine-acetic acid mixture, a 20-ml. portion of the distillate is poured into a graduated 25-ml. glass-stoppered cylinder. To this there are added 5 ml. of 20.0 N sodium hydroxide. After shaking vigorously, the cylinder is set aside for 15 minutes, during which time the liquid separates into two layers. The volume of the top layer is noted.

When this method was used it was found that the pyridine was not recovered in a pure state. Hence, it was necessary to find the relationship between amount of crude pyridine recovered—i.e., the top layer of liquid—and the amount of pyridine that was originally present in the mixture. Furthermore, the amount of crude pyridine recovered might be influenced either by the amount of acetic acid present or by the concentration of the sodium hydroxide used.

In order to determine this relationship and the effect of sodium hydroxide and acetic acid concentration, a quantity of reagent grade pyridine of such purity that it distilled between 114° and 116° C. was selected and two series of mixtures were prepared. The first series consisted of several mixtures of water and pyri-



dine, each of which differed from the other, in its pyridine concentration. The second series consisted of mixtures of water, pyridine, and acetic acid. In this series the acid concentration of each mixture was 0.3 *N* but the mixtures differed from each other in pyridine concentration. The pyridine concentration of each mixture was then determined by the above method. The mixtures were again analyzed in the same manner, except that 15.3 *N* instead of 20.0 *N* sodium hydroxide was used. The results obtained are shown in Table I and Figure 1.

#### DISCUSSION

The results in Table I clearly show that when the mixtures were 0.3 *N* with acetic acid, the amount of crude pyridine obtained was for all practical purposes the same as when no acid was present in the mixtures. Hence, it may be assumed that acetic acid in concentrations of 0.3 *N* or less will not affect the amount of crude pyridine recovered. When the concentration of the sodium hydroxide solution was 15.3 *N* instead of 20.0 *N*, a measurable difference was obtained in the amount of crude pyri-

dine recovered. Hence, the strength of this solution should be maintained at approximately 20 *N* as specified in the method. When this concentration is used, the volume figure for the top layer of liquid may be directly converted to per cent pyridine by means of the approximate curve in Figure 1.

#### SUMMARY

To determine the concentration of pyridine in distillates consisting of pyridine, water, and acetic acid, the pyridine, in an impure state, is separated by treating the distillate with strong sodium hydroxide. The relationship between this crude pyridine and the percentage of pure pyridine present in the distillate is then determined by means of a curve.

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## A Precision Head for Small Fractionating Columns

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THE most satisfactory head design for laboratory columns of all kinds is the total condensation partial take-off type originally reported by Loveless (1) and modified by Whitmore and Lux (5) and others. This design is not subject to the mechanical difficulties inherent in heads of the partial condensation total take-off variety (3, 5) and permits accurate regulation of

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the distillation rate. It is particularly adaptable for use on fractionating columns which utilize small samples, since the holdup is negligible if the head is properly constructed.

When such columns are operated under reduced pressure, there is a tendency for the distillate to dissolve the lubricant in the conventional stopcock, allowing air to leak in around the barrel and rise through the tiny pool of reflux liquid above the take-off tube. This situation prevents accurate adjustment of the distillation rate and at very low operating pressures (10 to 25 mm.) may cause air-locks which entirely prevent the removal of the distillate.

The head design shown in Figure 1 has been used by the author in these laboratories for several years. It was developed for

fractionating columns used in the purification of small samples of high-boiling hydrocarbons. The holdup is very small and no air-locks are formed even at operating pressures of 10 mm.

The needle valve (Figure 2) provides extremely accurate regulation of take-off rates of from 0.01 to 1.0 cc. per minute. Moreover, these rates are constant for long periods of time over a wide range of operating pressures. The small capillary in the valve seat acts as a siphon to promote a constant rate of removal of the distillate (2).

The valve bearing is made of 18-8 stainless steel machined to 0.8 cm. (5/16 inch) in diameter with a 1.25-cm. (0.5-inch) shoulder which rests on the short Pyrex tube. The upper face of this block is soldered to the bottom of a standard 0.3-cm. (0.125-inch) compression joint from which the lower threads have been removed. The finished bearing is drilled and tapped to receive the 8-32 thread on the valve spindle.

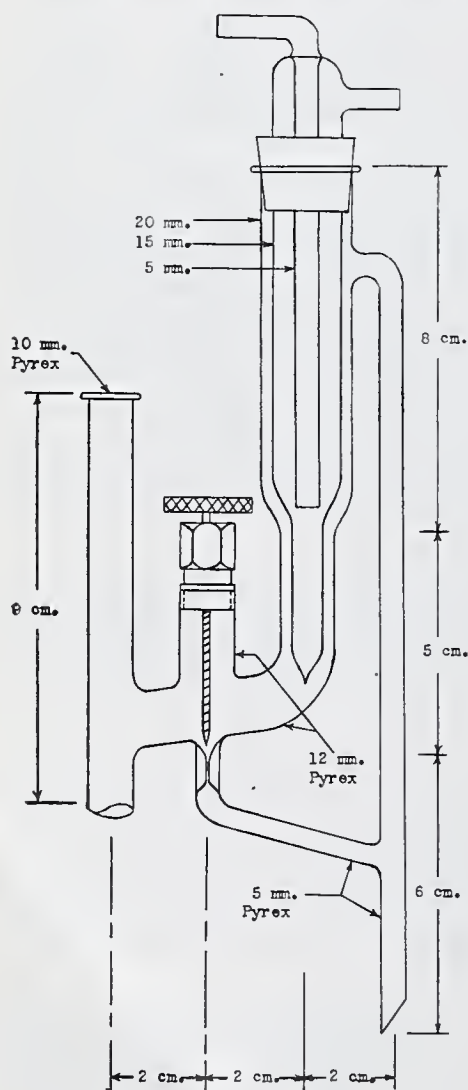


Figure 1. Head Detail

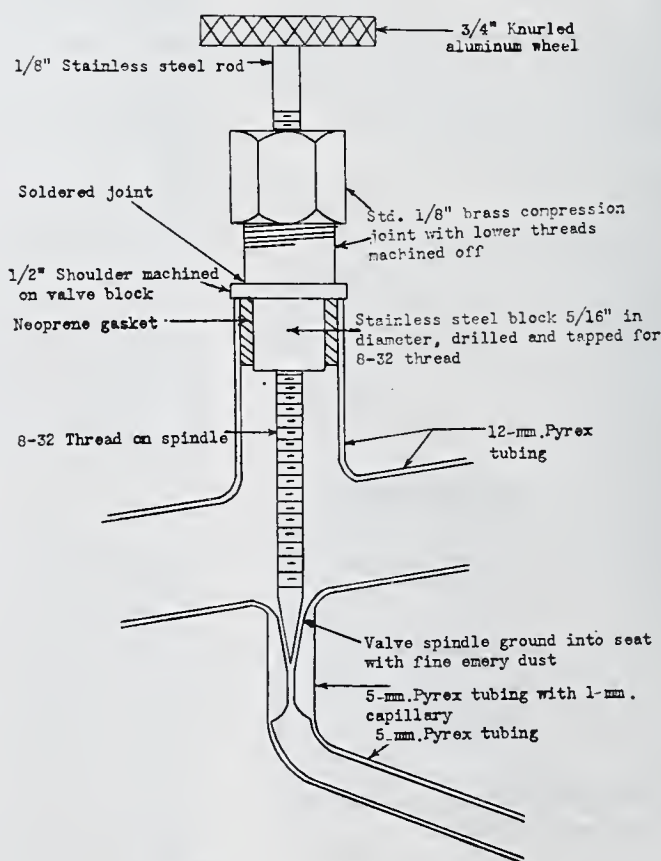


Figure 2. Needle Valve Detail



The needle-valve spindle is of 0.3-cm. (0.125-inch) stainless steel rod threaded to fit the valve bearing. A small knurled wheel is threaded to the top of the spindle. A fine point is round on the end of the valve spindle with a Carborundum wheel and the valve seat produced by grinding this point into the capillary tubing with a fine grade of emery dust.

Strands of absorbent cotton impregnated with a stiff grease are stuffed around the spindle in the depression of the bearing and compressed to an air-tight seal by the hexagonal nut. A small piece is cut from 0.3-cm. (0.125-inch) Neoprene tubing and slipped over the end of the valve bearing, so that the assembled nut fits tightly to the inside of the Pyrex tube. When properly packed and assembled, such a bearing will easily retain vacuums of 3 to 5 mm.

An efficient column for use with this head consists of a Pyrex tube, 8 mm. in inside diameter, providing 30 cm. of packed section. This inner tube is surrounded by a 35-mm. tube wound with Nichrome wire over asbestos spacer cords. The inner

tube and heating jacket is covered by a piece of 45-mm. Pyrex tubing which acts as an insulator. The inner tube was packed with Wilson helices (6) 0.24 cm. ( $\frac{3}{32}$  inch) in diameter. The column was operated at reduced pressures in conjunction with the fraction receiver described by Towne, Eby, and Young (4). This column proved to be very efficient in the purification of small samples (10 to 25 cc.). It had 14.5 theoretical plates at total reflux and an H.E.T.P. of 2.06 cm.

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## A Circulating Device for Use with a Hydrogen Electrode

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THE device described here has been found useful in connection with a hydrogen electrode in two circumstances: first, when the solution, whose hydrogen-ion concentration is being measured, contains a very soluble gas which would be carried away if the hydrogen were allowed merely to bubble through it. A resaturator may be used, but under certain conditions this is rather impractical. The second situation is when a deuterium electrode is desired. With the device described here macroquantities of the gas may be used, but an excessive amount is not required. These two conditions were present in measurements made by the authors on the second ionization constant of deuterio-carbonic acid. The results of these measurements have been reported elsewhere (1) but the circulating device has not been adequately described.

The construction of most of the apparatus is self-evident from the diagram. *V* is a Bunsen valve made from a medicine dropper

bulb. The proper size of slit can be found with a few trials and it may be inserted through the end of the wide tubing when the rubber stopper is removed. The mercury in the side arm is raised and lowered about 10 cm. by means of a motor and eccentric, raising and lowering a leveling bulb at a rate of about 15 times per minute. When the mercury rises the pressure in the adjacent part of the apparatus increases. Hence, the hydrogen bubbles through the solution around the platinum electrode until the pressure in the entire apparatus becomes uniform. When the mercury is lowered the hydrogen escapes through the Bunsen valve. Thus the hydrogen tends to circulate through the apparatus. During this period the stopcock of the cell, *S*<sub>2</sub>, should be kept closed. When an e.m.f. measurement is made the circulation may be stopped and the stopcock of the cell opened if desired.

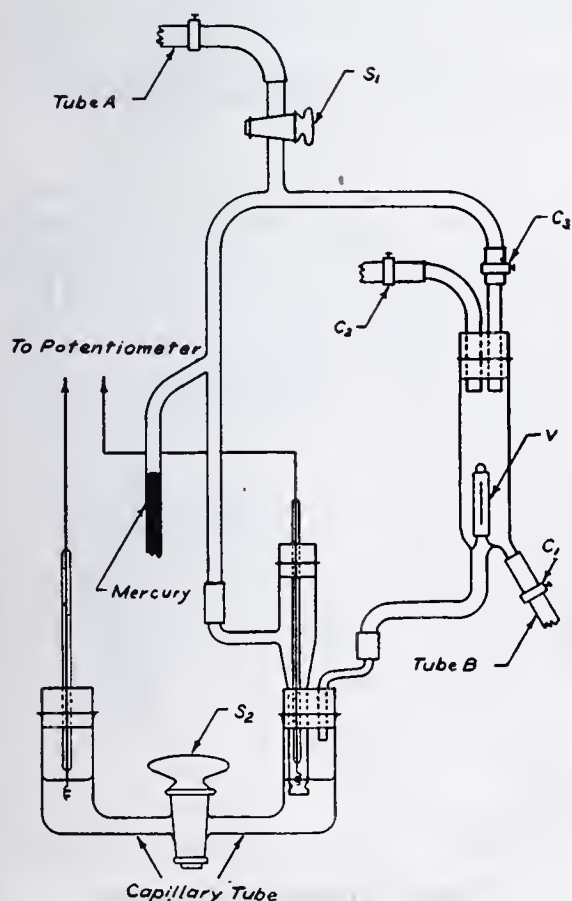
The apparatus is filled and flushed out by proper manipulation of clamps *C*<sub>1</sub>, *C*<sub>2</sub>, *C*<sub>3</sub>, stopcocks *S*<sub>1</sub> and *S*<sub>2</sub>, and the mercury column, hydrogen being admitted through tubes *A* and *B*. Admittance of dry hydrogen would alter the concentration of the solution slightly. This is ordinarily a very small error but may be avoided to a large extent by allowing the hydrogen to bubble through water, or better yet, through a sample of the solution before it is admitted to the apparatus through *A* and *B*. As a precautionary measure all rubber connections should be kept coated with collodion. In the authors' apparatus the volume, including the space in the cell above the solution, was 35 cc. For one determination about 100 cc. of hydrogen (deuterium) were used, the excess gas being used for flushing. It is usually most convenient to fill the device with hydrogen, so that when the mercury column is at its mean position the total pressure in the system is equal to that of the atmosphere. The corrections to apply in order to obtain the partial pressure of the hydrogen are obvious. In the authors' measurements, at equilibrium, the fluctuations in e.m.f. due to change in hydrogen pressure were less than 0.1 millivolt.

In order to avoid condensation it is necessary to keep the gas-phase portion of the apparatus at a temperature somewhat higher than that of the solution. This was accomplished by fastening the circulating device to a wooden block and suspending it in a small box in such a manner that the bottom of the box was slightly above the surface of the water in the thermostat in which the cell was immersed. When the thermostat was adjusted to 25° C. a current of air warmed to 30° C. was passed up through the bottom of the box through several holes. The hydrogen which circulates through the solution is also warmed to about 30° C. and this introduces a slight but unavoidable error.

This circulating device is obviously not limited to the particular type of cell depicted here. Harned and Scholes (2) have described in an extremely brief manner what is evidently an elaborate device for the circulation of hydrogen. Apparently their device is somewhat similar to the one described here.

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# Apparatus for Measuring Rate of Gas Penetration through Food-Packaging Materials

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An apparatus is described for measuring the rate of gas penetration through flexible materials. The apparatus is particularly suitable for determining the rate of oxygen penetration into pouches used for food packaging. The absolute accuracy of the measurements is determined mainly by the accuracy of gas analysis. The relative accuracy can be changed by varying the time of penetration and thus the difference between start and end concentration of oxygen in the gas inside the pouch.

A MAJOR factor in the spoilage of many dehydrated food products is the partial pressure of oxygen in the gas surrounding the food, particularly when the product contains fat. According to Holm, Schaffer, and Haller (5) butter oil containing a concentration of oxygen less than 0.5% by volume "will remain in good condition for a long storage period even under what would generally be considered severe conditions of storage". Some manufacturers, therefore, remove oxygen by evacuation; others replace air with an inert gas. Before the war, metal containers were used to package the product and ensure a low oxygen content over a long period. Because of the scarcity of metals these containers have been replaced by pouches constructed of cellophane or similar materials. No satisfactory method has so far been made available to determine how well such packages maintain the desired low partial oxygen pressure.

The rate of change of partial oxygen pressure in the gas inside a package is among the most important criteria for grading a container.

Elder (2), Shuman (8), and Todd (9) recently described manometers for measuring gas permeability of film materials without regard to changes in partial pressure. Shuman gives examples of measuring "air transmission". Air transmission thus determined, however, is no suitable criterion for judging packaging materials. An undesirable material with a high permeability for oxygen and a low permeability for nitrogen may show a lower rate of air transmission than a desirable material with low oxygen and high nitrogen permeability. The manometers might, of course, be used to measure separately the permeabilities of pure gases and thence to draw conclusions as to the probable transmission of these gases from mixtures such as air.

For testing packing materials and particularly pouches, however, the authors preferred to work out a method which determines the major criterion—namely, changes in partial oxygen pressure—directly, and is applicable especially in cases where no significant differences in total pressure are involved.

The rate of penetration by diffusion (dependent on differences of partial pressure only) can be determined by gas analysis or other means designed to measure oxygen concentration. Such studies have been made by several investigators. Krogh (7) designed a metal diffusion chamber to measure the diffusion rate of oxygen through animal membranes, from a gas to a liquid or from a liquid to a liquid. His equipment did not lend itself to the proposed studies. Harvey and Morrison (3) used cultures of luminous bacteria to determine oxygen concentration. According to them, the oxygen concentration just allowing perceptible luminescence is about 1 part by weight of oxygen dissolved in  $3.7 \times 10^6$  cc. of sea water. To obtain relative data on the rate at which oxygen penetrates various materials, Hill (4) used the luminous bacteria as an indicator. Alexeev and Matal'skiĭ (1) determined the diffusion rate of gaseous mixtures through

rubber membranes; but the present writers have been unable to obtain data on the type of apparatus or the method of analysis used.

## APPARATUS

The apparatus was designed mainly for measuring changes in the oxygen concentration of a gas separated from air by various packaging materials. These measurements are particularly valuable when mechanical flaws are absent.

Although different types of apparatus have been tested in this laboratory, only the one found satisfactory is described here (Figure 1). This consists of a glass diffusion chamber, *A*, having an outside diameter of 6 cm. and a length of about 18 cm. Gas may be introduced and sampled through a tube, *B*, the end of which is about 8 cm. below the top of the diffusion chamber. *B* connects to either a nitrogen tank or a gas-sampling bulb, through the three-way stopcock, *C*, and rubber tubing *D*. A glass receptacle, *E*, containing mercury is fastened to the diffusion chamber with a piece of bicycle inner tubing, *F*. The orifice, *G*, of chamber *A* may then be closed with a glass bell, *Q*, or with a sack of the packaging material in question. It has been desirable to hold the glass bell or packaging material in the mercury with

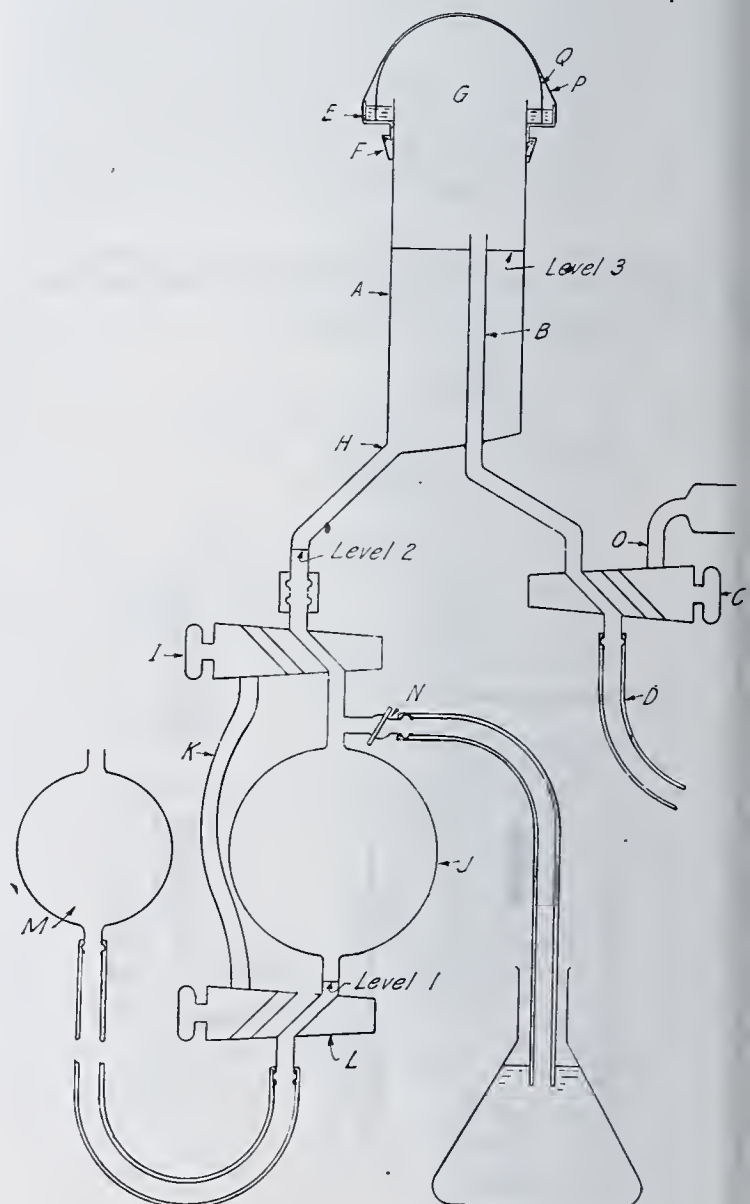


Figure 1. Diagram of Apparatus



Scotch tape, *P*, fastened over the top. Tube *H* is connected by a piece of rubber (pressure) tubing with a three-way stopcock, *I*. One opening of this stopcock is connected to the 250-ml. glass bulb, *J*, while the other connects with a glass tube, *K*. Thus by manipulating stopcocks *I* and *L*, connection to the diffusion chamber may be made either through bulb *J* or through tube *K*. Stopcock *L* connects with the mercury-leveling bulb by means of rubber tubing.

In operation, the mercury-leveling bulb, *M*, and stopcocks *I* and *L* are manipulated so that *K* is completely filled with mercury. This tube remains filled during measurements. The glass bell or packaging material is held solidly in the mercury seal by Scotch tape. *M* is adjusted so that the mercury stands at level 1. Stopcock *N* is opened, and stopcock *I* is turned to connect bulb *J* and chamber *A*. Stopcock *C* is opened into tube *B*, and gas is allowed to flow from a nitrogen tank through *A*. The excess gas escaping through *N* is allowed to bubble through water. Thus a constant positive pressure is maintained in the apparatus during replacement of the gas.

After the gas has flowed for a few seconds, *M* is raised so that the mercury rises from level 1 just to the top of *J*. The leveling bulb is then lowered, and the mercury allowed to fall to level 1. Meanwhile the nitrogen must be kept flowing fast enough to ensure constant escape of gas through *N* as measured by constant bubbling through the water seal. Two manipulations of the leveling bulb usually give satisfactory replacement of the air. Stopcocks *C* and *N* are then closed simultaneously, and the tube at the lower end of *C* is connected by rubber tubing to a gas-sampling bulb containing mercury. *C* is now opened into tube *B*, and the mercury in the gas-sampling bulb is forced up to flow into *O*. *C* is then opened into *B*, and *I* and *L* are adjusted to open the connection between *M* and *A* through *J*. *M* is raised, and simultaneously the mercury in the sampling bulb is lowered. This drives the gas from *A* into the sampling bulb.

When the mercury in the apparatus reaches level 2, stopcocks *C* and *N* are closed, and the sampling bulb is removed for analysis of the sample. *M* is then lowered; and *N* is opened, allowing all the mercury, except that above *I*, to flow back into the leveling bulb. This mercury may be used in other apparatus during the diffusion study.

At the expiration of the time allotted for diffusion, a second sample is taken. (Diffusion time must be determined according to the permeability of the material to be tested. As a rule, 48 hours is satisfactory.) For the second sample the gas-sampling bulb is connected and manipulated as before. *M* is raised to a point about level with the top of the diffusion chamber; *I* and *L* are adjusted so that the mercury flows into the diffusion chamber through *K*. As the mercury flows, the gas sample is collected in the sampling bulb. When the mercury reaches level 3 the stopcocks may be shut off and the gas sample removed for analysis. The mercury in the diffusion chamber is returned to the leveling bulb.

The gas has been analyzed with a modified Haldane apparatus described by Kleiber (6). The mean standard difference between two results on the same sample is below  $\pm 0.01\%$ .

## RESULTS

The rate of oxygen penetration can be calculated, assuming the amount of gas in the diffusion chamber to remain constant and considering the effect of changes in total pressure (barometric fluctuations) negligible in comparison with the effect of a difference of partial oxygen pressure of 0.2 atmosphere. The change in total amount of gas by the oxygen entering the chamber also is negligible, since the diffusion time is chosen so that the increase of oxygen concentration in the diffusion chamber does not exceed a few per cent.

Before using the apparatus for diffusion studies it was necessary to prove that samples of gas taken from the chamber would be comparable. To establish this point, opening *G* was closed with a glass bell, the apparatus was filled with nitrogen, and sample 1 was removed. As soon as possible (10 to 15 minutes), sample 2 was taken. The methods of sampling already described were followed. Typical results are as follows:

Run No.	Oxygen Concentration		Elapsed Time
	Sample 1 %	Sample 2 %	Min.
1	0.210	0.204	10
2	0.357	0.359	15
3	0.229	0.227	15
4	0.190	0.198	15
5	0.204	0.210	10
6	0.189	0.195	10

As a second check on the reliability of the diffusion equipment it was necessary to be sure there were no leaks. To test for leaks the glass bell was placed over opening *G*, and the apparatus was filled with nitrogen as before. A sample was secured, the equipment was allowed to stand, and then a second sample was taken:

Run No.	Oxygen Concentration		Elapsed Time
	Sample 1 %	Sample 2 %	Hours
1	0.173	0.178	48
2	0.210	0.209	48
3	0.220	0.223	22
4	0.187	0.186	48

Since the differences in samples 1 and 2 in these tabulations lie below the figure given by Kleiber as the standard error of the oxygen determination with the apparatus used, the diffusion chamber may be considered satisfactory, and the small discrepancies between samples may be ignored. Leaks have not been observed during the period of study (over 7 months), and thus routine checks before each diffusion study would not seem necessary.

To determine the rate of diffusion of oxygen one must know the volume into which the gas diffuses.

The diffusion chamber was filled with water, and the volume of water was then measured by draining the liquid to level 2 and allowing it to drain from tube *B* just to stopcock *C*. When diffusion rates were measured on pouches, the volume of the pouches was taken by calibrating with water. Since the open end of the sack is placed in mercury to a depth of 1.5 cm., the water level in calibrating is placed 1.5 cm. from the top of the sack. The total volume then is the sum of the diffusion chamber volume plus the volume of the sack.

Flexible sacks may be immersed in the mercury seal by attaching them to glass or metal rings with Scotch tape. Metal hooks on the top of rings permit the attachment of rubber bands, which submerge the juncture of sack and ring into the mercury. Flat pieces of flexible material have been crimped over the top of the diffusion chamber and held in place by a rubber band. This method may be criticized because it introduces folds and creases in the material. With the low rate of penetration observed in the authors' measurements, however, those irregularities in the surface areas can hardly affect the rate of exchange.

The following data, collected on a sack made of MSAT No. 480 laminated cellophane, will serve as an example of the results:

The total volume into which the oxygen diffused was 900 ml.; the area of sack exposed was 500 sq. cm.; the diffusion time was 555 hours; the temperature was 30° C. The oxygen content at 0 hours was 0.054%; at 555 hours, 0.175%. Thus the diffusion rate may be calculated as follows:

$$\frac{\text{Volume of gas in diffusion chamber and sack} \times \text{increase in } O_2 \text{ concentration}}{\text{Sack area exposed to air} \times \text{time}} = \text{rate/unit area/unit time}$$

$$\frac{900 \times (0.175 - 0.054)0.01}{500 \times 555} = 0.0000039 \text{ ml. of } O_2/\text{sq. cm./hour}$$

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# Use of an Alternating Current Solenoid in Freeze Tests

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NUMEROUS types of apparatus have been developed in the past two years to determine the relative flexibility and brittle point of synthetic materials at subzero temperatures. In employing any "freeze test" to judge the suitability of a material for low-temperature service, the mechanics of the test device used and the methods of conditioning the sample (time, temperature, etc., 2) should be considered in addition to the actual test data obtained.

In order to reproduce test results the velocity of the member inducing the deformation must be constant, since brittleness is a function of the rate of bending. Inability to reproduce test results is frequently due to lack of control of velocity, and hence of the energy supplied by the test device.

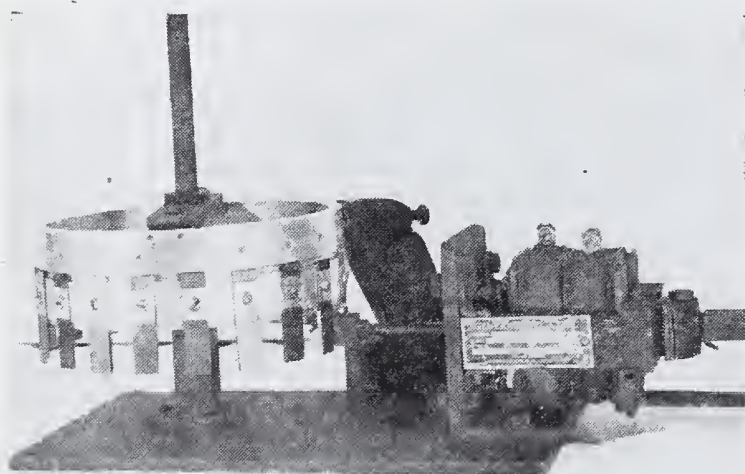


Figure 1

The bent loop test described by Martin (6) and tentatively adopted by A.S.T.M. (Designation D736-43T) is an excellent type of test for determining the relative flexibility of a series of test specimens, but the procedure for conducting the test fails to specify a specific rate at which the specimens are to be flexed. Most apparatus for performing this test employs either a crank and lever or a direct-push type of mechanism which is hand-operated, permitting some variation of the rate of flexing. The direct-push type is the simpler of the two devices and is the one more commonly used. The rate at which this push-type device can be operated depends upon the number and flexibility of the samples under test. Other flexibility tests, such as the bend test in which the specimen is bent through  $90^\circ$  over a 0.125-inch pin, or the bend test in which the mandrel size depends on the gage of the material being tested, are hand-operated. Thus the rate of flexing varies from test to test and from operator to operator, and variations observed in test results from laboratory to laboratory depend, to a large extent, on the human element involved in the test.

The weakness of most of the test devices which have been developed to determine the brittle point of synthetic materials is due also to the lack of controlled velocity. Bell Telephone Laboratories in presenting their hand-operated brittle point apparatus (8) stated that better test results were obtained if only one sample was under test at a time. Improved results were due to better control of velocity. This apparatus has been modified by Morris, James, and Werkenthin (7) to give better control of temperature and higher velocity at point of impact. Kemp, Malm, and Winspear (5) have fitted the original Bell apparatus with an electric motor and a set of gears in an attempt to control the rate at which the sample strikes the arm. Both groups of investigators have concluded that it is necessary to restrict an individual test to a single sample. The Bell Telephone Laboratories' apparatus which has been tentatively adopted by A.S.T.M. (D746-43T) operates with a quadrant having a peripheral speed of  $6.5 \pm 0.5$  feet per second.

Chatten, Eller, and Werkenthin (1) have developed a test device in which a swinging pendulum imparts a hammer blow to the test specimen. Graves and Davis (4) have presented an apparatus in which a spring-actuated hammer imparts the blow to the test specimens. The motorized apparatus, the pendulum device, and the spring-actuated hammer are very good from the standpoint of control.

Another brittle point test in which the specimen is subjected to a hammer blow was developed in the Goodrich Laboratories by Garvey (3). Its outstanding feature is economy of sample, and its main disadvantage is lack of control in imparting the hammer blow.

## ALTERNATING CURRENT SOLENOID AS A SOURCE OF POWER

An alternating current solenoid is a simple, efficient, easily controlled source of power which may readily be applied to many existing devices for measuring freeze resistance. It will supply energy at a reasonably constant rate, if it is operated at constant voltage. By varying the voltage, the speed at which the plunger of the solenoid travels may readily be controlled. A very simple way of controlling the desired voltage is by use of a Variac, an autotransformer which can easily be set to any desired output voltage.

The use of an alternating current solenoid as it has been applied to the Du Pont brittleness tester is shown in Figure 1. The brittleness tester consists of an aluminum drum from which the samples are suspended. The drum may be rotated from outside the working chamber in order to place the sample in front of the hammer. This hammer is made of linen impregnated with phenolic resin and is actuated by the solenoid. The leading edge of the hammer has been machined to a 0.0625-inch radius. The drum has twenty numbered slots from which samples,  $2 \times 0.5 \times 0.075 \pm 0.010$  inches, are suspended. It is counterbored above each slot and a spring-activated pin drops into this hole to ensure proper alignment of each sample with the hammer before testing. The lower edge of the slot, over which the samples must bend or break when struck by the hammer, has been machined to a  $1/2$ -inch radius. Each sample is held in place by a machine screw with a square washer. This apparatus is designed to operate in a cold, dry atmosphere (carbon dioxide).

The alternating current solenoid which is the source of power for the hammer is of the pull-type, having a 1-inch stroke, and is rated as having a 14-pound pull. This pull-type solenoid was converted to a push-type by drilling a 0.25-inch hole through the end of the frame and into the center of the cross section of the plunger. A brass rod on which the hammer is mounted was screwed into the end of the plunger. A second solenoid, much smaller in size, is used to return the hammer to its starting position. Both solenoids are individually controlled and are operated on a 110-volt 60-cycle circuit.

**TEST PROCEDURE.** The general test procedure when there are a large number of compounds to be tested is as follows:

Four test pieces,  $2 \times 0.5 \times 0.075 \pm 0.010$  inches, of each of five compounds are fastened to the drum with the machine screw just tight enough to hold the samples in place, the drum is put on the test stand, and the unit is installed in the cold box. After the desired temperature has been maintained for 1 hour the samples in slots 1, 5, 9, 13, and 17 are tested. When any one of these samples fails the remaining three pieces of the same compound are tested as checks. After exposure at the next lower temperature, the same procedure is repeated, starting with samples in slots 2, 6, 10, 14, and 18. Thus, one sample of each compound is tested at each temperature and when failure occurs the remaining untested pieces of the compound are tested. If all four test pieces of a compound are tested without failure the procedure is repeated, starting with the first test piece of that group at the next lower temperature.

In general, the test is started at  $-30^\circ$  F. and the temperature is lowered in  $5^\circ$  F. intervals with 1-hour exposure at each temperature to ensure temperature equilibrium. The test unit is adjusted so that the hammer travels approximately 0.25 inch (depending on thickness of sample) after contacting the test piece.



Table I. Du Pont Brittleness Test Data

Hammer striking sample 0.25 inch below bottom edge of drum and traveling 0.25 inch after making contact)

Compound	Temperature of Brittle Point, ° F.				
Buna S	-90°	-90°	-90°	-90°	-90°
Neoprene Type GN	-65°	-65°	-65°	-65°	-65°
Neoprene Type FR	-75°	-70°	-70°	-75°	-75°
Rubber	-65°	-65°	-60°	-65°	-65°

and strikes it 0.25 inch below the edge of the drum, thus producing an bend of approximately 45°, as is shown in Figure 2. The reproducibility of the brittle point for a given compound is excellent. When failure occurs the sample breaks off squarely at the lower edge of the drum.

Table I shows the results obtained with the Du Pont brittleness tester on four compounds which were tested five different times over a period of 3 weeks.



Figure 2

This apparatus may also be used to determine the low-temperature flexibility of coated fabrics. This test may be conducted by replacing the present drum with one that has a longer skirt on which loops of the material to be tested are mounted. After being conditioned at the desired temperature, each loop is compressed by the hammer against the drum until the distance between hammer and drum is equal to twice the gage of the fabric. Figure 3 shows an isometric sketch of a proposed brittleness test apparatus employing an alternating current solenoid of the fish-type.

The basic design of this apparatus is similar to the Bell Telephone Laboratories' (4) motorized apparatus, but the energy to drive the sample at a constant rate is supplied by an alternating current solenoid instead of a motor. This solenoid is rated as producing a 5-pound push at a maximum stroke of 1 inch. The side dimensions of the tank containing the immersion medium are 15 × 4.5 × 6 inches, and sixteen specimens, 1.50 × 0.25 × 0.075 ± 0.010 inches, may be mounted on the sample holder. A movable bar, not shown in the figure, supports the specimens in a horizontal position during the conditioning period. It is moved to the front of the tank before testing the specimens. The edge of the hammer that strikes the sample has a 0.0625-inch radius, which is the same as the radius on the arm used in the Bell Telephone Laboratories' apparatus. The position of the sample holder on which the specimens are mounted may be varied by changing the size of the inserts at the supports. As may be seen in Figure 3, the structure supporting the solenoid is hinged, in order that it may be swung back to simplify the operation of removing and replacing the specimen holder. The sample holder is equipped with small eye-bolts, so that hooks may be used in removing and replacing the holder. Alignment of the solenoid with the test specimen is assured by a latch which drops into the notches shown on the upper rod supporting the solenoid. The immersion medium, usually methyl alcohol, ethyl alcohol, or acetone, is cooled by circulation through a coil placed in an acetone and dry ice bath. The immersion medium inlet is at the

DU PONT BRITTLNESS TESTER  
(LIQUID MEDIUM TYPE)

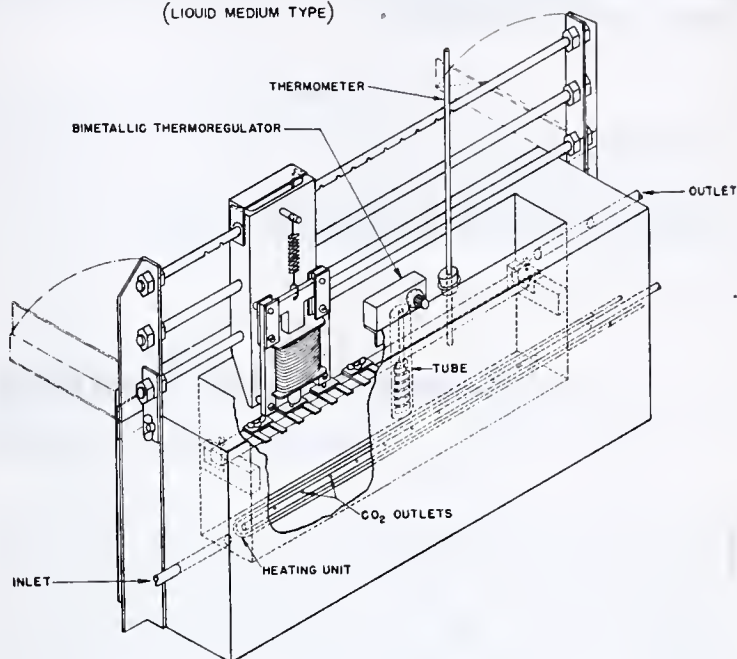


Figure 3

bottom of the tank on the left end and the outlet is at the top on the right end. The tube shown along the bottom of the tank is for the purpose of bubbling carbon dioxide through the medium in order to give mild agitation and to produce a carbon dioxide blanket over the medium to reduce fire hazard. In case fumes from the immersion medium constitute a health hazard, the apparatus should be operated under a hood.

The pump used to provide circulation of the immersion medium may be controlled by a bimetallic thermoregulator located in the tank as shown. The bimetallic thermoregulator is mounted in a tube in which a nonvolatile liquid, such as a low-temperature hydraulic oil, is used to prevent volatile vapors from entering the chamber which houses the contact points where arcing may occur. A condenser placed in the circuit with the bimetallic thermoregulator and the motor of the pump will reduce the arcing to a minimum and help retain the sensitivity of the thermoregulator. The temperature of the immersion medium is indicated by the thermometer which is mounted next to the bimetallic thermoregulator. The heating unit enables one to warm up the immersion medium rapidly.

The velocity at the instant of impact of the hammers for both the 14- and 5-pound solenoids was determined and found to vary linearly from 7 to 9 feet per second when the voltage was varied from 100 to 120 volts. Therefore, when the solenoids are operated at rated voltage (110 volts) the velocity of the hammers at the instant of impact is 8 feet per second.

The time of travel of the hammers from the point of zero velocity to the point of impact is very short; hence the velocity at impact is affected by the phase of the voltage cycle at which the solenoid becomes energized. This seems to indicate that a more scientific test unit might be obtained if a direct current solenoid were used. However, the use of a direct current solenoid is not only impracticable in many cases, but is believed to be unnecessary. It was found experimentally with the unit which is operated in air that the kinetic energy imparted by the solenoid at rated voltage was 3.5 times the kinetic energy necessary to break the samples. In view of this fact, the slight variation in the kinetic energy resulting from energizing the solenoid at various phases of the voltage cycle is negligible.

#### ADVANTAGES

Operation is simple and efficient. The human element has been eliminated as much as possible from the test.

A variable number of samples may be tested at one time. Stocks of any hardness or those swollen by solvents can be



tested. The velocity at impact and the rate of bending of the samples are reasonably constant.

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PRESENTED before the Division of Rubber Chemistry, AMERICAN CHEMICAL SOCIETY, at its New York meeting.

## Monel Metal Pouring Plate for Silica Fusions

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IN MANY analytical laboratories, particularly in the mining and metallurgical industries, a large number of silica fusions are carried out daily. These are usually made in platinum crucibles or dishes, using sodium carbonate or a mixture of carbonates as a flux. Fusion may be made on the sample directly or on the insoluble portion after prior acid treatment.

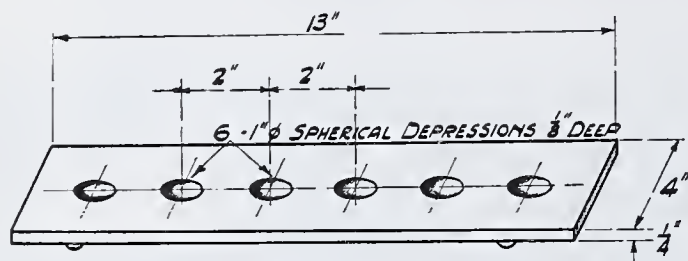


Figure 1. Pouring Plate

Since these determinations are generally used for control purposes, speed is essential. If the fusion is allowed to solidify in the crucible, a long time is required to dissolve the contents in the dilute hydrochloric acid usually employed. The practice of allowing the melt to cool on the sides of the crucible requires careful manipulation, and even then most of the sample solidifies at the bottom. When solution of the solidified melt is slow, more hydrochloric acid is often added to hasten the action. However, as the resulting solution usually must be evaporated to dryness to remove silica, the increase in bulk prolongs the evaporation period. When a fusion reacts slowly with acid, or adheres to the crucible, there is a temptation to hasten solution by scraping with a glass rod, which usually results in ultimately deforming or injuring the platinum. The time lost in waiting for cooled fusions in platinum crucibles to dissolve is particularly important to those laboratories where an abundant supply of platinum ware is not available.

This difficulty can be overcome by pouring the molten fusion into a depression on a Monel metal plate. The material quickly solidifies in the form of a flat button which is transferred with platinum-tipped tongs to a beaker or casserole, together with the platinum crucible. A few milliliters of dilute hydrochloric acid suffice to dissolve the thin film of solidified melt left in the platinum crucible or dish, which can be washed and removed for another fusion in a minute or two. Owing to its surface, the button rapidly goes into solution in dilute acid. The chilling effect produced by pouring on the Monel plate gives a product which dissolves more readily than a fusion allowed to cool in the crucible. If necessary, solution of the button may be aided by breaking up with a flat-tipped glass stirring rod.

This procedure was first brought to the writer's notice some time ago in the laboratories of the International Nickel Company of Canada at Copper Cliff, Ontario, where it had been in use for many years. It has since been applied successfully to a wide variety of products.

The plate is illustrated in Figure 1. Molten sodium carbonate under these conditions has no effect on Monel metal. Plates are still in perfect condition after hundreds of thousands of fusions have been poured on them. Tests for copper and nickel with pure carbonate fluxes have shown that the quantities of these elements picked up from the plate are below the limit of quantitative determination. The extreme resistance of Monel to molten sodium carbonate does not extend to peroxide or bisulfate, and fusions of the latter should not be poured onto such a plate. The Monel plate can be cleaned and polished occasionally with any standard metal cleaner and rinsed thoroughly in water to remove traces of polish.

The plate illustrated in Figure 1 is intended for general analytical work where 0.5- to 2.0-gram samples are fused with 5 to 10 times their weight of carbonate flux. Where other quantities are employed the size of the depression may be altered slightly. The object should be the formation of a slightly rounded button which will fill the depression but not overflow to give a thin layer on the plate. In the latter case the thin edge of the button may break in several pieces when picked up with the tongs. A slightly larger plate to contain two rows of depressions may be used where a large number of fusions are carried out as routine determinations.

Remove the crucible from gas burner or electric furnace when contents have fused, pour onto plate, place crucible on Transil or Alberene stone table top or on the plate itself, pour the next crucible, place the first solidified button in the corresponding crucible, and transfer to beaker or casserole. It is not good practice to put a red-hot platinum crucible on a Monel plate but after the crucible is poured it is almost invariably cool enough to place directly on the plate beside the button if desired. Transfer the button from the crucible into the beaker and add a little dilute hydrochloric acid to the crucible. Continue pouring in this manner, wash, and remove the crucibles. While the major portion of the fusion contained in the button is dissolving, the platinum crucibles or dishes may be used for further fusions.

## Spectrophotometry

Two papers have been prepared by the Research Department of the Calco Chemical Division, American Cyanamid Co., and are available through the Advertising Department, Bound Brook, N. J.

"Spectrophotometry and the Colorist", *Calco Technical Bulletin* 756, prepared by E. I. Stearns, discusses interpretation of spectrophotometric data and suggests methods of application to mill production and research problems.

"Identification of Organic Pigments by Spectrophotometric Curve Shape", *Calco Technical Bulletin* 754, prepared by R. Abbott and E. I. Stearns, illustrates the general method of approach to the problem of identification of organic pigments by their characteristic absorption curve shape.



# A Simplified Conductometric Titration Apparatus

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THE advantages and disadvantages of conductometric titrations are too well known to be detailed here. It is often advantageous to titrate mixtures or turbid solutions conductometrically, and this method has been used in this laboratory for the analysis of complex mixtures encountered in research on the reproofing of cotton cloth. However, the complicated apparatus ordinarily used discourages more general application of the method. The apparatus described by Whittemore and his co-workers (3, 4, 5) is perhaps the simplest. In their method the voltage is adjusted to a constant value after each addition of titrating solution and the current passing through the conductivity cell is measured. By plotting milliliters of solution added against milliamperes, a typical conductometric curve is obtained.

This apparatus and procedure have been further simplified in this laboratory by the use of a constant-voltage transformer and an alternating current milliammeter of low internal resistance, as diagrammed in Figure 1, considering the circuit at *M* completed through *A*.

The innovation, though simple, makes the conductometric titration very easy, since it is only necessary to read one meter, compared with the former procedure involving the adjustment of fluctuating voltage with a potentiometer, reading this on a voltmeter, and simultaneously reading the milliammeter.

The constant-voltage transformer,  $T_1$ , is the type generally used for 8-volt lamps in such instruments as the Coleman spectrophotometer and thus is generally available. Its cost is less than that of the meter, transformer, and potentiometers it replaces. The more common 115-volt constant-voltage transformer with an auxiliary step-down transformer is equally suitable and this combination may be substituted for  $T_1$ . Along with the constant-voltage transformer it may be necessary to have an additional constant load to meet its minimum load requirement and avoid overheating.

Because of war conditions, the sale of meters is restricted, and, unfortunately, an alternating current milliammeter is more rarely used than other types in a chemical laboratory. A low-range alternating current voltmeter is nearly always available, however. In this laboratory, the 2.5-volt range of an alternating current meter of the rectifier type, 1000 ohms per volt, was used instead of a milliammeter as follows:

A radio-type transformer,  $T_2$ , commonly known as an audio-output transformer, with an impedance ratio of about 500 to 1 (turn ratio of 22 to 1), was used as a current transformer to convert the relatively high current at a small voltage drop to a much higher voltage, and impress this voltage on the meter. It is then possible to read the current directly from the meter in relative units. If actual values are desired, the factor may be obtained from a consideration of the meter and transformer constants, or by direct calibration. This circuit is shown in Figure 1, considering the circuit at *M* completed through *B*. The transformer to be used as  $T_2$  should be selected to reflect the resistance of the meter as 5 ohms in series with the cell—for example, to use the components cited above, a transformer matching 2500 ohms to 5 ohms was used. In general, satisfactory results are obtained using a value within the limits of 4 to 8 ohms for the low-impedance winding.

Electrodes are conveniently made by welding platinum disks to platinum wire and sealing each into appropriately shaped glass tubes as shown in Figure 1. The size of the electrodes and the distance between them are chosen to give a convenient initial conductance. For example, in the titration of approximately 100 ml. of 0.001 *N* solution with 0.01 *N* reagents, disks 1 cm. in diameter and spaced about 2.5 cm. apart were used. In the titration of approximately 100 ml. of 0.01 *N* solutions with 0.1 *N* reagents, disks 0.3 cm. in diameter and spaced about 3 cm. apart were used. It was found that the apparatus described was generally suitable for cell-electrolyte combinations which resulted in resistances of the order of 400 to 10,000 ohms. It was not found necessary to

extend the range in the direction of lower resistances, as this could be circumvented by titrating with more dilute solutions.

The limit of accuracy of conductometric methods, given by Kolthoff and Sandell (2) as 0.5 to 1%, is readily attained with reasonable precautions:

The solution being titrated should be uniformly stirred with a glass stirrer (not a metal one), but a "whirlpool" should not be allowed, since the addition of the titrating medium will change the shape of the vortex and cause an irregularity in the curve.

The beaker and electrodes should not be moved once the titration is begun.

The two transformers (if  $T_2$  is used) must be placed with their cores at right angles or sufficiently distant from each other to avoid inducing current in  $T_2$ .

The concentration of the reagent solution should be at least 10 to 20 times that of the solution to be titrated in order to obtain rectilinear graphs.

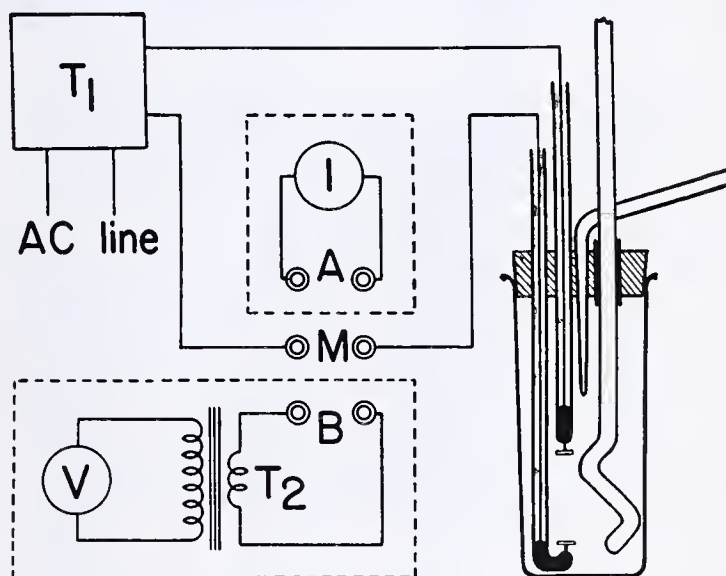


Figure 1. Diagrams of Simplified Conductometric Apparatus

$T_1$ , 8-volt constant-voltage transformer (or combination of 115-volt constant-voltage transformer and 115- to 8-volt step-down transformer)

*M*, To be completed through either circuit *A* or *B*

Circuit *A*, *A*, metering circuit using milliammeter *I*  
*I*, low-resistance 0- to 30-milliamperes alternating current meter

Circuit *B*, *B*, metering circuit using  $T_2$  and voltmeter *V*  
 $T_2$ , audio-output transformer  
*V*, 0- to 2.5-volt alternating current meter, 1000 ohms per volt

Graphs of conductometric titrations obtainable with this apparatus are of the same type and precision as shown by other authors (1, 3, 4, 5). The text by Britton (1) is particularly useful for interpretation of the graphs obtained.

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# An Improved Timing Siphon

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CYCLIC processes can best be controlled by the use of a clock motor or other similar device. However, a substitute for a mechanical timer is often needed. In this laboratory the common siphon proved unsatisfactory for such use because after the first delivery of water, the siphon tube remains full of bubbles. In this case, the siphon vessel may never again fill up, the water siphoning out as fast as it runs in, or the siphon may operate when the vessel is only one-fourth or one-half full.

This difficulty is overcome in the design given in Figure 1.

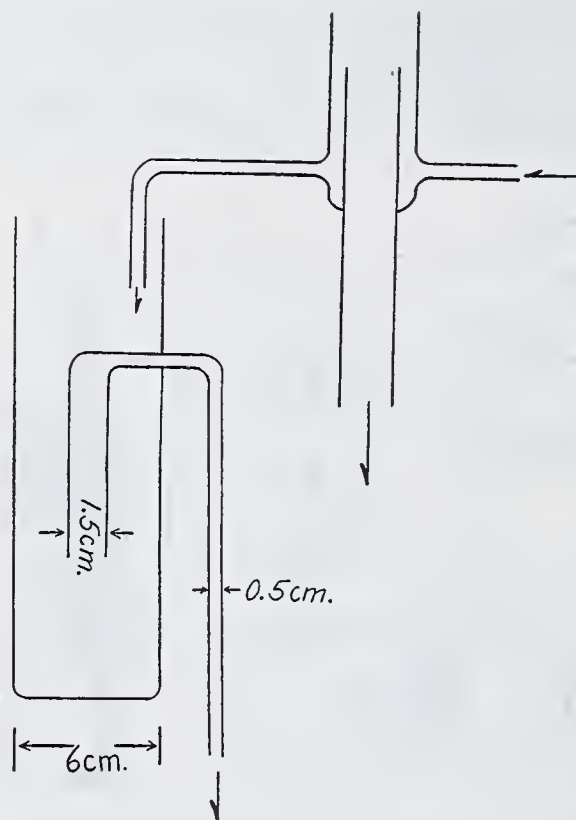


Figure 1

The central tube is 1.5 cm. in diameter and joins on to a 5-mm. tube. This large central tube completely breaks up the bubble string and gives cycles which are accurate to 2 or 3% if the water temperature does not vary too greatly. The size of the outer vessel can be varied to any size, while the central tube and the draining tube can be enlarged to any degree, provided the ratio of sizes is not made smaller and the draining tube does not become too large actually to function as a siphon. A conventional constant-head device furnished a constant flow of water to the vessel containing the siphon.

The conductivity of the water can be used to work the control mechanisms as follows: Two electrodes which are intermittently bathed by the water can be connected to a source of power and a suitable relay which in turn can actuate pumps, valves, lights, etc. A satisfactory relay circuit is described by Rudy and Fugassi (1).

Liquids other than water can be used with this device, with due regard for abnormal viscosity or for vapor pressure which might cause bubble formation due to the lower pressure at the top of the siphon tube.

In place of conductivity control, a photoelectric control may also be used.

Present address, Mine Safety Appliances Co., Pittsburgh, Pa.

By controlling the rate of influx of water, the rate of draining, with the siphon, and the placing of the electrodes, almost any length of cycle can be obtained.

## LITERATURE CITED

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# Simple Automatic Pump for Collecting Gases at Low Pressures

I. E. PUDDINGTON

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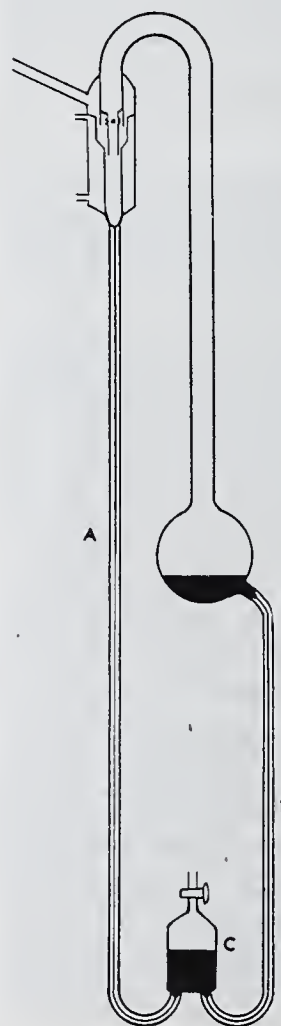
IN A particular piece of work it was found desirable to maintain a reaction mixture at low pressure and at the same time to collect evolved gases. Since the reaction was being followed for extended periods, a manually controlled pump such as a Toepler was inconvenient. The following simple design was found to be efficient and required virtually no attention during long periods of operation.

The details of the design are shown in the figure. It consists essentially of a mercury diffusion pump in which the compressed gas, instead of being removed by a mechanical pump in the usual way, is entrained by the condensed mercury, as in the ordinary Sprengle pump, carried down the capillary tube, A, and discharged into the gas holder, C. A second capillary, B, returns the mercury from the gas holder to the boiler.

The height of the diffusion pump above the mercury boiler must be sufficiently great to keep the hydrostatic pressure of the mercury and entrained gas in A always greater than the mercury alone in B. In the present design, which operated successfully on systems where the pressure was as high as 3 cm. of mercury, this height is about 40 cm. Pumping speeds should be greater as this head is increased. In order that the collected gas should not occupy too large a volume, the gas holder should be located about 4 cm. below the level of the mercury in the boiler.

Capillary tubing of 2-mm. diameter is satisfactory for the top half of A, while the use of 1-mm. tubing in the bottom half greatly reduces the tendency for compressed gas bubbles to move up the tube. A heating element of about 60-watt capacity wrapped about the tube delivering mercury vapor to the jets reduces bumping in the boiler and eliminates excessive refluxing of the mercury.

This pump is particularly useful for collecting gases at low pressures. Its limited capacity makes it rather slow at higher pressures. The design described reduced the pressure in a 350-cm. volume from 35 to less than  $10^{-5}$  mm. in 3 hours. A lowering from 1 to  $10^{-5}$  mm. was obtained in the same system in 1 minutes.



ISSUED as N.R.C. No. 1216.



# Determining Volatile Bases in Fish

## Comparison of Precision of Certain Methods

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Methods were investigated for determining total volatile base and tertiary volatile base in fish flesh as an index of spoilage. Sampling methods tested included use of press juice, protein-free press juice, 60% ethanol-leached samples, samples "liquidized" with 60% ethanol, and samples of ground fish suspended directly in solution. Volatile base was removed by microdiffusion, distillation, and aeration. Most precise results were obtained for total volatile base by extracting the fish flesh with 60% ethanol and removing the volatile base by distillation from the solution made alkaline with borax. Tertiary volatile base could best be determined by a slight modification of the microdiffusion method of Beatty and Gibbons (3) whereby a sample extracted with 60% ethanol was used in place of press juice.

THE determination of volatile bases in fish has been widely used as an index of the freshness of fish. A considerable number of procedures have been suggested, and since results obtained by the different methods are not always in agreement, the comparative results obtained by some of them have been studied. These were procedures which obtain the total volatile base by separation from alkaline solution into an excess of standard acid or which determine tertiary amines by separation in the presence of formaldehyde.

### SAMPLING TECHNIQUE

An experimental lot of fish of varying degrees of freshness was prepared as follows: Fifty-five eviscerated silver salmon were procured when 4 hours out of the water. Twenty fish were stored at room temperature and 30 in crushed ice. Five were dressed immediately and the flesh, free of skin and bones, was ground twice through an electric meat grinder, mixed thoroughly, and packed in 0.2-kg. (0.5-pound) cans. The sealed cans were immediately frozen and stored at about  $-15^{\circ}\text{C}$ . ( $5^{\circ}\text{F}$ ). At suitable intervals the salmon stored at room temperature or in ice were sampled by removing five fish and treating as above. It was assumed that no increase in volatile base occurred after freezing, and the cans of frozen fish were used for subsequent tests. (All tests were completed within 6 months; additional samples stored 3.5 years showed an average increase in total volatile base of 18%. Greatest decomposition occurred in the fresher samples, where significant changes may have occurred during the 6 months' storage period.)

The method of bringing the volatile base into solution prior to distillation into standard acid is of importance in obtaining accurate results. If both volatile base and other nitrogenous substances are present during distillation, the latter may break down to give added volatile base. Low results may be obtained if an attempt is made to extract the volatile base from the protein or if the protein is removed from solution by precipitation. In the first case all the base may not be extracted and in the second case it may be lost by being adsorbed on the protein precipitate.

In obtaining press juice for the tests, the ground fish flesh was placed in a canvas cloth and pressed in a Carver laboratory press at a pressure of 8000 to 10,000 pounds per square inch (562 to 703 kg. per sq. cm.). Since the fish had been frozen, relatively large volumes of press juice were obtained (100 to 200 ml. of juice per kg. of flesh). The press juice ordinarily was clear, but became cloudy upon standing. When made alkaline for the distillation and particularly in the presence of formaldehyde, it frequently gelled, especially if from stale fish. With fresh fish the juice usually became very cloudy, and sometimes considerable sediment settled out, but as a rule it did not solidify.

A series of tests was made with press juice from which the protein had been precipitated by trichloroacetic acid. Twenty-

five milliliters of the juice were treated with a few milliliters of a saturated trichloroacetic acid solution in a centrifuge tube. After centrifuging, the clear solution was decanted into a 100-ml. volumetric flask, and the voluminous precipitate was washed three times with 10-ml. portions of water. The combined solution and washings were made up to 100 ml. for the determination. The solution was clear and little or no sediment separated either upon standing or when made alkaline.

The exceedingly gummy nature of the precipitate formed when trichloroacetic acid was added to the press juice made washing difficult, and doubtlessly considerable of the volatile base was left adhering to the precipitate. In some instances the precipitate was more gummy than in others, making for irregular adsorption of volatile base. A more easily washed precipitate could be obtained by precipitating diluted press juice, but so high a dilution was required (ten times or more) that the resulting sample did not contain enough volatile base to give reproducible results.

Extractions of the fish both with water and with 60% ethanol as described for meat by Allen (1) were tried. Preliminary tests indicated that for fish, as for meat, much more consistent results were obtained by means of the ethanol extractions; so all tests were made in this way. Forty grams of fish were stirred mechanically with 60 ml. of 60% ethanol, in a 250-ml. centrifuge bottle, the solution was centrifuged, and the clear solution decanted into a volumetric flask. The stirring and centrifuging were repeated with two more 75-ml. portions of 60% ethanol and the combined leachings were made up to 250 ml. The final solution was clear and did not solidify upon being made alkaline.

Another series of tests was run in which the fish was finely disintegrated in 60% ethanol by means of a liquidizer. (Several brands of this type apparatus were tried. Of those used, the Waring Blendor with special aluminum container gave the best results. This special container had a screw-type lid which prevented loss of solution during operation and gave quantitative results. With equipment in which the lid merely sat upon the top of the vessel, an appreciable loss of solution sometimes occurred.)

Forty grams of fish and 100 ml. of 60% ethanol were liquidized for 5 minutes, after which time the fish was completely disintegrated and formed a stable suspension from which the solid could be removed completely only by centrifuging. The solids remaining in the centrifuge tube were washed with two 25-ml. portions of 60% ethanol and the combined solution and washings were made up to 250 ml. The resulting solution was clear immediately after preparation but a fine precipitate usually formed after a short time giving a cloudy appearance.

### SEPARATION OF VOLATILE BASE

Determinations were run using the Conway and Byrne (4) microdiffusion technique as modified for fish by Beatty and Gibbons (3), and also using press juice freed of protein by trichloroacetic acid treatment. In applying the microdiffusion technique improvised glassware was used in place of the regular Conway dishes which were not readily available. A 5-ml. beaker for holding the standard acid was placed inside a Stender dish (60 mm. in diameter and 28 mm. deep). In order to assure that the lids of the Stender dishes in all cases fit tightly, it was necessary to regrind them with Carborundum and use a fairly heavy coating of stopcock grease. The ratio of surfaces of alkaline test sample and of standard acid exposed to total volumes is less in this improvised equipment than in standard Conway dishes but preliminary experiments showed that by increasing slightly the time of standing and temperature of incubation, recovery of ammonia from both pure ammonium chloride solution and standard ammonium chloride added to fish solutions was adequate (recovery consistently over 90%, usually better than 95% from solutions 0.001 *N* or stronger).

Samples prepared by extraction with alcohol were also dis-



tilled at atmospheric pressure. The presence of the alcohol during the distillation materially aided in reducing foaming. An excess of borax was used in place of magnesium oxide because it not only reduces hydrolysis of nitrogenous substances by virtue of its lower pH, but also, being denser than magnesium oxide, has less tendency to cause foaming. This distillation procedure is a considerable improvement over the ordinary aqueous distillations using magnesium oxide and a battery of six or more can easily be run with little or no attention.

Aeration was carried out in the apparatus exactly as described by the A.O.A.C. (2) procedure for meat, in which case finely ground fish flesh was suspended in water. In other cases fish flesh was liquidized with 60% ethanol and the resulting solution made alkaline and aerated without removal of fish particles. Aeration units were set up in batteries of six units in parallel and some difficulty was encountered in obtaining uniform aeration. This difficulty was overcome by use of capillary tubing of appropriate lengths as entrance tubes into each of the six tubes containing the fish samples. By adjusting the length of this tubing the pressure could be equalized, permitting uniform aeration in all samples. Four such batteries of six units were run at once.

An aeration period of 5 hours was used in all cases. Further aeration gave a slight increase in volatile base but this increase

continued indefinitely as long as the aeration was carried out, and probably represented a breakdown of the protein or other nitrogenous substances. All determinations were run in triplicate to allow for discarding occasional samples which did not receive adequate aeration when the inlet tubes to the sample vessels became clogged with fish flesh. Little or no difficulty was encountered with tubes becoming clogged when samples prepared in the liquidizer were used. In these cases the fish, being of a much finer state of subdivision, did not settle out as long as the aeration continued.

Tertiary volatile base was determined in each case by addition of neutral formaldehyde to the final solution of fish. The difference between the amount of standard alkali used in the determination and the blank was taken as equivalent to the volatile base present.

#### DISCUSSION OF RESULTS

Fish stored at room temperature for 1 day were still reasonably fresh but were slightly stale after 2 days (see Tables I and II). Accordingly, if total volatile base or tertiary volatile base is to be used as an index of spoilage a large increase would be anticipated between fish stored for 1 and 2 days. With the fish

Table I. Individual Determinations and Averages for Total Volatile Base in Silver Salmon<sup>a</sup>

Sample No.	Storage Conditions of Fish	Milligrams of Nitrogen per 100 Grams of Fish or per 100 Milliliters of Press Juice							
		Microdiffusion				Distillation		Aeration	
		Press juice	Protein free press juice	Leached with 60% ethanol	Liquidized with 60% ethanol	Leached with 60% ethanol	Liquidized with 60% ethanol	Suspended in water (A.O.A.C.)	Liquidized
1	Fish 4 hours out of water. Fresh	11.2	9.5	4.0	14.3	7.2	11.8	7.3	17.3
		11.6	6.5	3.9	14.7	12.9	12.0	7.2	17.1
		13.8	..	..	..	..	..	..	15.4
		Av.	12.2	8.0	14.5	10.1	11.9	7.3	16.6
2	1 day at room temperature. Fresh	11.7	7.3	5.3	11.9	13.0	11.8	9.7	16.4
		13.1	11.8	5.1	12.6	13.3	12.1	9.4	16.0
		11.9	7.3	..	..	13.1	11.5	..	15.8
		..	..	..	..	12.2	11.9	..	..
		Av.	12.2	8.8	12.3	12.9	11.8	9.6	16.1
3	2 days at room temperature. Slightly stale	18.0	15.6	12.4	24.3	16.5	20.6	17.7	26.8
		18.8	14.3	12.3	23.6	20.8	21.2	11.0	27.2
		17.6	..	..	..	21.6	21.3	..	25.4
		18.7	..	..	..	..	21.6	..	..
		Av.	18.3	15.0	24.0	19.6	21.2	14.4	26.5
4	3 days at room temperature. Very stale	28.0	23.1	19.2	42.8	34.8	31.0	37.0	40.7
		24.2	19.0	21.0	47.3	32.2	31.4	26.3	25.5
		15.6	..	..	46.8	29.4	31.4	35.6	39.6
		24.7	..	..	45.4	..	31.7	..	..
		Av.	23.1	21.0	45.6	32.1	31.4	33.0	35.3
5	4 days at room temperature. Putrid	39.8	35.0	35.4	44.2	47.0	35.4	28.2	48.0
		38.0	32.0	34.2	47.5	44.5	36.2	28.0	48.0
		35.4	..	..	..	..	35.3	27.2	46.7
		35.7	..	..	..	..	36.4	..	..
		Av.	37.2	33.5	34.8	45.9	35.8	27.8	47.6
A	3 days in ice. Fresh	12.6	12.3	..	13.1	12.1	11.1	7.6	14.6
		13.0	10.8	..	13.3	12.9	12.1	10.4	12.6
		13.5	..	..	..	11.5	11.3	8.9	15.2
		12.0	..	..	..	14.1	12.1	..	..
		Av.	12.8	11.6	..	13.2	11.7	9.0	14.1
B	6 days in ice. Fresh	13.3	7.6	12.7	13.3	12.6	13.3	4.7	15.3
		11.0	8.2	13.3	13.1	12.0	13.5	4.9	15.7
		14.2	..	..	..	13.6	..	10.1	..
		13.3	..	..	..	13.6	..	..	..
		Av.	12.9	7.9	13.0	13.2	13.4	6.6	15.5
C	10 days in ice. Slightly sweet odor	11.4	9.0	10.9	15.9	11.7	12.0	14.9	15.3
		12.6	11.3	10.5	16.8	11.6	12.1	12.2	13.7
		11.0	8.8	..	..	12.4	..	9.9	15.7
		12.5	11.1	..	..	12.1	..	..	..
		Av.	11.8	10.1	10.7	12.2	12.1	12.3	14.9
D	13 days in ice. Slightly stale	15.4	13.2	12.8	19.9	19.1	17.4	22.4	21.1
		18.3	9.7	14.4	20.6	19.5	17.7	14.6	23.8
		18.7	..	..	..	19.5	..	16.7	22.7
		15.4	..	..	..	17.4	..	..	..
		Av.	17.2	11.5	13.6	20.2	17.6	17.9	22.5
E	15 days in ice. Stale	18.3	18.6	21.7	19.9	23.0	19.5	27.4	27.7
		20.3	16.9	19.7	21.1	22.7	19.8	22.4	27.5
		24.4	18.8	..	..	..	..	20.1	27.6
		20.0	..	..	..	..	..	..	..
		Av.	21.2	18.1	20.7	22.9	19.7	23.3	27.6
F	17 days in ice. Very stale to slightly putrid	91.0	26.4	27.1	30.4	33.5	30.9	17.7	38.6
		34.0	17.6	29.0	30.8	33.3	30.8	17.2	39.0
		21.7	..	..	..	..	..	..	36.8
		Av.	48.9	22.0	28.1	30.6	30.9	17.5	38.1

<sup>a</sup> Each determination reported was started from beginning of sampling and was carried out separately, so that differences in results are due to combined sampling errors, errors in separations, and titration errors.



stored in ice a more gradual deterioration took place. After 6 days the fish were still fresh, but after 10 days, although not at all stale, they had developed a slightly sweet odor. After 13 days in ice the fish were slightly stale. Corresponding increases in volatile base content were found with both lots of fish, and larger or smaller increases were obtained by all the methods of analysis.

SAMPLING METHODS

Errors due to sampling methods would be most apparent as poor precision when several determinations were run on the same lot of fish. Of the five sampling methods used, outstandingly high reproducibility was obtained by use of the "liquidizer". Precision was uniformly high by this method, and there can be little doubt but that of the methods tried it gives by far the most homogeneous sample, which is also readily adaptable to subsequent steps of the determination.

Use of press juice, while simpler and somewhat less time-consuming than the use of the liquidized sample, gave very poor precision especially when working with stale samples. This is

believed to be caused by the tendency of press juice from such samples partially to solidify when made alkaline. Leaching the fish by stirring the flesh (previously ground in a meat grinder) with several portions of 60% ethanol gave good precision; this method is very time-consuming, but can be used if a liquidizer is not available.

METHODS OF REMOVING VOLATILE BASE

In preliminary tests the three methods of separating volatile base gave good precision when used on pure solutions in absence of fish. Errors occurring in the presence of fish were due largely to decomposition of protein, or other nitrogenous compounds, such as trimethylamine oxide during the separation from the alkaline solution. The results obtained did not indicate any outstanding advantage for any one method in all cases.

MICRODIFFUSION. This procedure has two advantages. First, it is convenient, especially with respect to saving in time, since many tests can be run simultaneously and little attention is required during the separation. The second advantage lies in the low temperature which can be maintained during separa-

Table II. Individual Determinations and Averages for Tertiary Volatile Base in Silver Salmon<sup>a</sup>

Sample No.	Storage Conditions of Fish	Milligrams of Nitrogen per 100 Grams of Fish or per 100 Milliliters of Press Juice							
		Microdiffusion		Distillation		Aeration		Suspended in water (A.O.A.C.)	Liquidized
		Press juice	Protein free press juice	Leached with 60% ethanol	Liquidized with 60% ethanol	Leached with 60% ethanol	Liquidized with 60% ethanol		
1	Fish 4 hours out of water. Fresh	0.00	0.23	0.00	0.13	0.00	1.00	0.21	0.51
		0.00	0.00	0.04	0.10	0.00	1.10	0.21	0.51
		0.30	..	..	..	..	..	0.21	0.51
		Av.	0.10	0.02	0.12	0.00	1.05	0.21	0.51
2	1 day at room temperature. Fresh	0.27	0.00	1.13	0.17	1.21	0.70	0.39	0.22
		0.00	0.38	1.05	0.10	0.72	0.67	0.29	0.73
		0.00	..	..	..	0.70	..	0.34	0.44
		0.64	..	..	..	0.77	..	..	..
		Av.	0.23	0.19	1.09	0.85	0.69	0.34	0.46
3	2 days at room temperature. Slightly stale	3.6	1.75	4.3	2.3	8.2	8.1	5.2	9.4
		3.2	4.2	4.2	2.2	8.2	8.7	5.4	9.2
		2.7	..	..	2.3	8.2	8.5	4.4	9.7
		..	..	..	2.4	..	..	..	..
		Av.	3.17	2.98	4.25	8.2	8.4	5.0	9.4
4	3 days at room temperature. Very stale	9.5	4.1	6.6	3.5	13.4	13.3	5.1	11.9
		6.8	2.5	6.9	3.5	12.4	13.8	5.1	11.7
		0.73	..	..	3.7	12.7	13.3	..	12.1
		3.7	..	..	3.8	..	..	..	..
		6.5	..	..	..	..	..	..	..
5	4 days at room temperature. Putrid	Av.	5.4	3.3	6.75	12.8	13.5	5.1	11.9
		12.4	6.1	8.0	3.9	15.3	14.7	6.5	13.6
		10.9	4.1	8.5	3.8	15.4	14.2	8.2	13.4
		8.3	..	..	3.9	..	..	11.1	..
		8.0	..	..	4.0	..	..	..	..
A	3 days in ice. Fresh	Av.	9.9	5.1	8.25	3.9	15.4	8.6	13.5
		0.26	0.00	0.12	0.25	0.61	1.00	0.40	0.31
		0.32	0.78	0.00	0.28	0.61	1.30	0.49	0.31
		0.75	..	..	0.07	0.42	..	0.40	..
		..	..	..	0.05	0.30	..	..	..
B	6 days in ice. Fresh	Av.	0.44	0.39	0.06	0.16	0.49	1.15	0.43
		0.88	0.50	0.60	0.35	1.30	1.30	0.45	1.14
		0.20	0.45	0.60	0.38	1.30	1.05	0.45	1.14
		0.22	..	..	..	1.30	..	..	..
		..	..	..	..	1.11	..	..	..
C	10 days in ice. Slightly sweet odor	Av.	0.43	0.48	0.60	0.37	1.25	1.18	0.45
		0.90	0.28	0.80	0.80	2.00	2.3	1.29	1.87
		0.83	3.2	0.70	0.70	2.00	2.1	1.24	1.87
		0.25	1.38	..	..	1.97	..	..	..
		0.58	..	..	..	..	..	..	..
D	13 days in ice. Slightly stale	Av.	1.55	..	..	..	..	..	..
		0.82	1.62	0.75	0.75	1.99	2.2	1.27	1.87
		3.3	2.4	3.6	1.28	5.8	5.2	4.2	5.9
		1.5	3.3	3.0	1.25	5.0	5.3	4.0	5.7
		1.7	..	..	..	5.0	..	3.8	6.1
E	15 days in ice. Stale	Av.	2.94	2.85	3.3	1.27	5.2	5.25	4.0
		3.9	..	..	..	4.8	..	..	..
		4.3	..	..	..	5.5	..	..	..
		..	..	..	..	..	..	..	..
		..	..	..	..	..	..	..	..
F	17 days in ice. Very stale to slightly putrid	Av.	3.76	4.93	4.3	1.93	5.7	6.85	6.45
		5.0	4.5	3.6	2.2	5.3	6.7	6.3	7.4
		1.49	4.5	5.0	1.67	6.0	7.0	6.6	7.5
		2.0	5.8	..	..	..	..	..	7.4
		5.8	..	..	..	..	..	..	..

<sup>a</sup> Each determination reported was started from beginning of sampling and carried out separately, so that differences in results are due to combined sampling errors, errors in separations, and titration errors.



tion, which assures a minimum decomposition of nitrogenous compounds.

Disadvantages are a lack of high precision, necessity of special equipment, and the need for great care in cleansing glassware and in making titrations. The lack of precision is due to errors in titration which occurred even when using a microburet because of the very small sample size. A fairly high dilution of the fish is required if a liquidizer is employed, owing to the relatively large volume of liquid needed to operate this equipment. In practice it was found that a concentration of fish corresponding to only about 150 grams per liter could be prepared and only 0.3 gram of fish is present when 2 ml. of this solution are used. With fresh samples having low volatile base content, such a small sample impairs precision.

**DISTILLATION.** Standard Kjeldahl distillation apparatus can be used, results can be obtained in a very short time, and very high precision is readily obtained, owing to the larger fish sample used (up to 40 grams per titration).

Disadvantages include the necessity of watching the distillations to prevent foaming, and the high results obtained for the tertiary volatile base determinations where values up to ten times as high as by the other procedure were found with fresh samples. The distillation procedure seems to be most suitable for determining total volatile base but it cannot be used for determining the tertiary bases unless allowance is made for the higher results obtained, especially with fresh fish.

**AERATION PROCEDURE.** Aeration is carried out at room temperature, so that a minimum of decomposition of nitrogenous constituents takes place. However, since no attempt is made to remove such nitrogenous material as is done with the other methods, even a slight decomposition of the large concentration of these interfering substances may be more serious than in the other methods. This method has the advantage of requiring a minimum of time to prepare the sample, since the centrifuging and washing steps are eliminated. Disadvantages include use of special equipment, long aeration time, and need for constant attention during aeration to prevent clogging of the aeration tubes. This method is rather cumbersome and is not recommended, although reasonably precise results are obtained.

#### RECOMMENDED PROCEDURE

Forty grams of fish are placed in a liquidizer (preferably with a tight-fitting lid) with 100 ml. of 60% ethanol and mixed for 5 minutes. The contents of the liquidizer are transferred

quantitatively to a 250-ml. centrifuge bottle, using 60% ethanol as wash solution, centrifuged for 10 minutes, and decanted into a 250-ml. volumetric flask. The solids in the centrifuge bottles are stirred with 25 ml. of 60% ethanol, centrifuged, and decanted into the volumetric flask, and the washing and centrifuging repeated with a second 25 ml. The volume is made up with 60% ethanol.

For the tertiary volatile base determination, a 2-ml. aliquot is pipetted into the outer section of a Conway dish, 2 ml. of neutral formalin solution are added, 1.00 ml. of 0.005 *N* hydrochloric acid is pipetted into the center dish, and then with the lid in place except for a small opening for the pipet, 1 ml. of saturated potassium carbonate solution is added from the quick-draining pipet. The lid, previously well greased at the ground-glass section, is quickly slid into place, the contents of the dish are mixed by a slight rotary motion, and the dish is incubated for 3 hours at 40° C. Blanks are run simultaneously in exactly the same way except for substituting 2 ml. of 60% ethanol for the fish solution. After incubation the excess acid is titrated, using a microburet and a mixed indicator, either methyl red-methylene blue or methyl red-bromo cresol green. The indicator solution should first be adjusted to the neutral point by the addition of dilute acid or alkali. Determinations should be carried out in triplicate.

The same procedure can be used for determining total volatile base except for the omission of added formalin, and use of 1.00 ml. of 0.020 *N* acid in the center of the Conway dish. However, the following distillation procedure is preferred by the authors because of the advantages previously mentioned:

The contents of the volumetric flask (after aliquots for tertiary volatile base have been withdrawn) are transferred to a 500-ml. Kjeldahl flask and 4 glass beads and 5 grams of powdered borax are added. The flask is quickly connected to the distillation equipment and 100 ml. of distillate are collected in 50 ml. of 0.05 *N* hydrochloric acid. If great difficulty should be encountered with foaming, as sometimes occurs with very stale samples, a few drops of caprylic alcohol may be added, but an excess should be avoided. A blank should be run simultaneously, using 60% ethanol in place of fish solution. Excess acid in the distillate is titrated with standard alkali, using methyl red as an indicator.

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## Polarographic Use of Organic Reagents Magnesium with 8-Hydroxyquinoline

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CARRUTHERS (2) has described a polarographic method for the determination of magnesium by reduction of the 8-hydroxyquinoline in a phosphate buffer solution of the precipitated magnesium inner complex salt. The present work is concerned with the determination of magnesium by polarographic measurement of the excess 8-hydroxyquinoline without removing the precipitate and avoids the difficulties which arise from the necessity for filtration.

#### APPARATUS AND MATERIALS

A Leeds & Northrup Electrochemograph equipped with the cell arrangement described by Furman, Bricker, and Whitesell (3) was used for the polarographic measurements. The work was done at room temperature (23° to 26° C.).

8-Hydroxyquinoline obtained from Paragon Testing Laboratories was recrystallized three times from ethanol-water mixtures. The standard solution contained 0.5 gram per liter in 5% ethanol.

The ammonia-ammonium chloride buffer (0.25 *M* in total ammonia, ammonium chloride approximately 0.036 *M*) was made from polarographically pure ammonium chloride and c.p. ammonium hydroxide and was adjusted to pH 10 with a Leeds & Northrup pH meter.

A standard magnesium solution (100 mg. per liter) was made by dissolving the appropriate amount of magnesium metal containing 0.1% maximum impurity in the smallest amount of 0.01 *M* hydrochloric acid and diluting the solution to the proper volume.

Other chemicals used were of analytical reagent grade tested for magnesium. In most cases it was absent.

The capillary had the following characteristics:  $m = 0.6695$  mg. per second,  $t = 4.55$  seconds at 1.0 volt against the satu-



A method for the determination of magnesium by the polarographic estimation of the excess 8-hydroxyquinoline left after precipitation of the magnesium salt is reported. The only major interfering cation which is common is titanium. The solubility of magnesium 8-hydroxyquinolate in ammonia-ammonium chloride buffer of pH 10 is  $1.9 \times 10^{-6}$  mole per liter. The method can be applied to estimation of magnesium in water and plant materials.

rated calomel electrode (S.C.E.) in the buffer solution; the head of mercury was 41.9 cm. Oxygen was removed by passing purified nitrogen through the solution for 15 minutes. All polarograms were taken at  $1/10$  sensitivity unless otherwise indicated.

#### METHOD

The method is based on the decrease in wave height of a given concentration of 8-hydroxyquinoline by the precipitation of part of it by magnesium in a buffered solution at pH 10 without removal of the precipitate.

In all this work 25-ml. volumetric flasks were used, unless some other size is indicated. Five milliliters of the standard 8-hydroxyquinoline solution and 10 ml. of the buffer were placed in the flask. For the original concentration, the flask was filled to the mark, mixed well, and the polarogram taken. For the precipitation, a given amount of the standard magnesium solution or of the unknown was added to the buffer and 8-hydroxyquinoline in the flask. The flasks were filled to the mark, mixed well, and shaken at frequent intervals for 1 to 2 hours, depending on the amount of magnesium. The polarogram was then taken. Figure 1 shows the character of the wave when the amount of magnesium changes with the same original concentration of 8-hydroxyquinoline in each case.

The polarogram consisted of two waves (Figure 2). The first wave had an  $E_{1/2} = 1.39$  volt vs. S.C.E. which did not shift appreciably with concentration in the range under consideration. The second wave had an  $E_{1/2} = 1.61$  volts vs. S.C.E. This half-wave potential shifted slightly with concentration and also considerably with slight changes in pH and so was not investigated further. The height of the wave that had  $E_{1/2} = 1.39$  volt was proportional to the concentration, as Table I shows. The decrease in wave height due to precipitation by the magnesium was proportional to the amount of magnesium present, and gave a straight-line calibration curve between 5 and 200 micrograms of magnesium in the 25-ml. volumes used.

#### SOLUBILITY OF MAGNESIUM 8-HYDROXYQUINOLATE

A small amount of moist magnesium 8-hydroxyquinolate prepared by precipitation in the usual way was washed with dilute ammonia and water until the washings were free of chloride and were colorless. Ten milliliters of the buffer were diluted to 25 ml. and saturated by intermittent shaking for 12 hours in contact with some of the moist preparation. A polarogram was taken at  $1/5$  sensitivity. The wave height corresponded to a concentration of  $1.9 \times 10^{-6}$  mole per liter of magnesium 8-hydroxyquinolate, which is equal to 46 micrograms of magnesium per liter. The maximum error due to the solubility of the precipitate is 1 microgram of magnesium in 25 ml. This error is in general smaller because of the decrease of the solubility of the precipitate due to the excess of 8-hydroxyquinoline.

**INTERFERENCES:** Lundell and Hoffman (5) list the cations precipitated by 8-hydroxyquinoline. Any cations that are precipitated under the conditions used will interfere and must be removed or converted into complexes which are not precipitated. Electrolysis with the Melaven cell (5, 6) removes the common interfering ions except aluminum, titanium, and calcium. 25 to 50 mg. of ammonium tartrate will keep in solution 150 micrograms of aluminum in the 25-ml. volumes used. No reagent was found that would keep titanium in solution; this must be re-

moved by precipitation as the hydroxide when present. Calcium can be tolerated in amounts up to 0.5 mg. in 25 ml. with no interference.

#### DETERMINATION OF MAGNESIUM IN TAP WATER

The tap water available is the type obtained from limestone and dolomite beds. The iron content is low (0.6 p.p.m.) and with the size of sample taken causes no interference.

**PROCEDURE:** Fifty milligrams of ammonium tartrate were dissolved in 10 ml. of the buffer and 5 ml. of the standard 8-hydroxyquinoline solution were added. A 5-ml. sample of the water was added and diluted to the mark. After 2 hours' shaking and standing, the polarogram was taken. The results are shown in Table II.

#### DETERMINATION OF MAGNESIUM IN PLANT MATERIALS

Samples of tobacco obtained from the Connecticut Agricultural Experimental Station and of dried pine seedling cuttings obtained from Ray Dawson of the Biology Department, Princeton University, were analyzed.

**PROCEDURE:** Samples were dried, ashed, and dissolved in dilute sulfuric acid. The resulting solution was electrolyzed with the Melaven cell and filtered to remove the small amount of black precipitate due to the manganese. The filtrate was made up to some standard volume and an aliquot taken such that about 100 micrograms of magnesium were present. The procedure as for water determination was followed (Table III).

#### DISCUSSION

The strength of the standard 8-hydroxyquinoline solution is not too critical, but 0.5 gram per liter fits the procedure best.

Table I. Constancy of  $I_d/c$  with  $C$  for 8-Hydroxyquinoline

$C$ , Moles/Liter $\times 10^4$	$I_d$ , Microamperes, Corrected for $I_r$	$I_d/c$
0.276	0.28	1.01
0.552	0.56	1.01
0.690	0.70	1.01
1.379	1.38	1.00
2.070	2.08	1.01
2.758	2.76	1.00
	Av.	1.01

Table II. Analysis of Tap Water

Magnesium Present, P.P.M.		
Polarographic 5.2	Colorimetric (4) 5	Gravimetric <sup>a</sup> (1) 5
<sup>a</sup> Weighed as magnesium 8-hydroxyquinolate.		

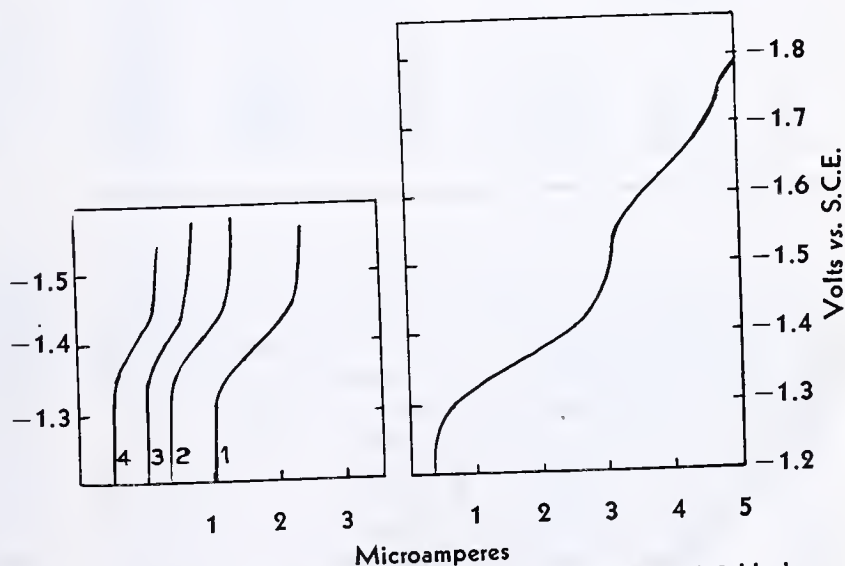


Figure 1. Effect of Magnesium on 8-Hydroxyquinoline  
 $C = 1.379 \times 10^{-4}$  mole per liter  
Magnesium: (1) none, (2) 2 micrograms per ml., (3) 4 micrograms per ml., (4) 3.8 micrograms per ml.

Figure 2. Polarogram of 8-Hydroxyquinoline  
 $C = 2.758 \times 10^{-4}$  mole per liter at pH 10



**Table III. Determination of Magnesium in Plant Materials**

Sample No.	Weight of Sample Gram	MgO Found %	MgO Reported <sup>a</sup> %
Tobacco			
1	0.2020	1.15	1.24
1	0.1987	1.17	...
2	0.1959	1.45	...
2	0.2045	1.39	1.54
3	0.2318	1.58	...
4	0.1898	1.76	1.59
4	0.1956	1.70	1.70
Pine Seedling Cuttings			
	0.3360	0.14	...
	0.3437	0.14	0.12 <sup>b</sup>
	0.3769	0.12	...

<sup>a</sup> MgO determined by gravimetric separation and determination of Mg as pyrophosphate.

<sup>b</sup> Spectroscopic value. Sample CaIa (7).

The solution decomposes slightly with time and a blank has to be run each time it is used. The decomposition can be followed

polarographically, but at the present time the decomposition products are not known.

The precipitation has to be carried out at room temperature because ammonium 8-hydroxyquinolate is too volatile even at 60° C. Ammonium tartrate has no effect on either  $I_r$  or  $I_d$ , and hence the calibration data are good for solutions containing small amounts of tartrate.

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## NOTE ON ANALYTICAL PROCEDURE

### Use of a Color Indicator in the Tannin Method for Determination of Beryllium and Aluminum

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THE accuracy of the tannin method, as modified by Nichols and Schempf (2), for the separation and determination of beryllium and aluminum depends on an accurate control of the pH during precipitation of the aluminum. The only means now available for obtaining this control is the pH meter, an instrument very difficult to obtain at the present time. Because of the strategic importance of these metals a search for a suitable color indicator seemed advisable.

In order to determine the value of this mixed indicator in the analysis, a series of determinations was made on a solution mixture of aluminum and beryllium sulfates of known concentration, the results of which are shown in Table I.

Although in each analysis the Beckman pH reading was taken, no further adjustment of the acidity was made. The accuracy obtained compares favorably with that of Nichols and Schempf.

**Table I. Use of Color Indicator in Analysis**

(Al<sub>2</sub>O<sub>3</sub> taken, 0.0743 gram; BeO taken, 0.0753 gram)

No.	Beckman pH Reading	Al <sub>2</sub> O <sub>3</sub> Obtained Gram	Error Mg.	BeO Obtained Gram	Error Mg.
1	4.50	0.0747	+0.4	0.0750	-0.3
2	4.58	0.0747	+0.4	0.0752	-0.1
3	4.68	0.0748	+0.5	0.0748	-0.5
4	4.69	0.0745	+0.2	0.0758	+0.5
5	4.62	0.0743	±0.0	0.0750	-0.2

Of the numerous indicators tried, a mixture of 1 drop of methyl red to 6 drops of bromocresol green (0.1% solutions) per 500 ml. of the buffer solution prescribed by Nichols and Schempf, was found satisfactory if the color change is approached from the basic side as described below. To the solution, diluted to 500 ml. and containing the buffer and indicators, ammonia (1 to 1) is added until the solution assumes a blue-green color, indicating a pH well above 5. Dilute (6 N) sulfuric acid is then added slowly and with constant stirring. The following color changes are noted: blue-green, blue, purple, reddish purple, red. The first appearance of the reddish purple was found to coincide very closely with a pH of 4.6, the pH necessary for the complete separation of the two metals. The color change is definite and easily distinguished. If desired, however, it may be checked against the Clark and Lubs buffer mixture (1) having a pH of 4.6.

## LITERATURE CITED

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- (2) Nichols, M. L., and Schempf, J. M., *IND. ENG. CHEM., ANAL. ED.*, **11**, 278 (1939).

### Fifteen-Year Collective Index for Analytical Edition

A fifteen-year collective index of the ANALYTICAL EDITION of INDUSTRIAL AND ENGINEERING CHEMISTRY, complete through 1943, is being prepared by Charles L. Bernier, associate editor of *Chemical Abstracts*, with the expectation of being able to issue it early in 1945, as a pamphlet of the same page size as regular issues of the ANALYTICAL EDITION, if sufficient paper for printing can be obtained.

Both subject and author indexes are to be included, following in general the practice of *Chemical Abstracts*, but with certain variations suggested by the special nature of this analytical index.

Present plans contemplate furnishing copies of the index at a nominal price to any subscriber to the ANALYTICAL EDITION who places his order before publication, and selling copies after that time at a somewhat higher price. At present no definite price can be quoted, as it will depend somewhat upon the number of copies printed. It will be very helpful if those wishing to receive the index will notify Walter J. Murphy, Editor, 1155 Sixteenth St., N. W., Washington 6, D. C., preferably prior to October 1.



## A Needed Improvement in Baking Control Methods for Organic Finishes

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Numerous variables, as discussed, introduce large errors in the control of short-time organic enamel baking operations. A relatively simple baking control method is described which compensates for these variables and gives comparable enamel film properties under widely varying baking conditions. It is based on enamel film or metal temperature determinations and the calculation of consequent reaction velocity. An instrument is also described which automatically makes the necessary temperature measurements and calculations, and continuously indicates the percentage completion of the baking operation, according to predetermined standards.

THE introduction during recent years of industrial organic finishes designed to bake at short, relatively high-temperature schedules emphasizes the need for more accurate methods of controlling baking operations. The long-established method of placing the metal to be baked in an oven, set at a given temperature, for a predetermined period of time gives satisfactory results when the baking time is in the order of one hour or longer. However, in the case of appreciably shorter bakes, for which higher temperatures are employed, serious variations are encountered in the enamel film temperature resulting from variables which are difficult or impossible to control. These variables include heating characteristics of the oven, mass of material in the oven, mass-surface area relationship of the painted ware, etc. They all have an effect on the baking speed of the enamel film and result in serious variations in the properties, such as color, hardness, water resistance, and durability of films baked according to predetermined time-oven temperature schedules.

Experimental evidence shows that the speed of curing or polymerization of an organic enamel film, like the speed of other chemical reactions, is a function of temperature. Furthermore, there is ample evidence to indicate that the temperature of an enamel film on a metal substrate follows very closely the temperature of the metal surface, regardless of the heat gradient between the metal and the surrounding air bath. The metal is a good conductor of heat and the air is a very poor one; hence any heat gradient at the paint film-metal interface is quickly neutralized because the heat is conducted away from or to the paint film by the metal. It follows that an accurate measurement of the surface temperature of the metal is also an indica-

tion of the temperature of the paint film. Such a measurement can be made with a contact thermocouple.

A copper-constantan thermocouple junction is brazed directly to the sample of ware to be baked, or, for the sake of simplicity it can be brazed to a square of thin sheet copper which is then clamped to the face of the painted metal sample in such a way that it is in intimate contact with the surface. A section of the sample is first wiped clean of the wet paint film. If the clamp is light in weight and if it is insulated from the metal with asbestos cloth, its added mass will not influence the temperature measurements appreciably, and any error by comparison with the former method of brazing directly to the metal object will be negligible. The temperature measurements are made by means of an ordinary laboratory potentiometer with a scale reading directly in degrees Fahrenheit.

Employing such a means of determining the temperature of the metal and of the paint film, a curve has been plotted (A, Figure 1) which shows the temperature-time relationship for a sample of metal in a laboratory box-type oven, the air temperature of which was controlled at 260° F. The sample consisted of 6 square feet of 26-gage sheet steel weighing approximately 4.5 pounds and painted on one side only. B represents the temperature rise for an equal area of painted 14-gage steel, weighing approximately 30 pounds, during a second run in the same oven. The correct baking time for the 26-gage metal to give paint film properties which have been arbitrarily set up as standards for the particular finish under consideration is 66 minutes. By inspection of the curve it is seen that, although the 14-gage metal lags somewhat behind the 26-gage for the first 20 or 30 minutes, it approaches very closely the temperature of the latter for at least 60 to 65% of the baking time. As a matter of fact, after 66 minutes of baking, the color, hardness, flexibility, etc., of the enamel film on the two metal samples are equal within the experimental limits of the testing methods.

This illustrates the fact that various weights of ware having varying thicknesses within reasonable limits can be baked for a predetermined period of time in the neighborhood of an hour or longer with little, if any, variation in resulting paint film properties. In shorter baking operations, however, this is not the case.

Curve A, Figure 2, shows the temperature rise for the 4.5 pounds of 26-gage steel and B represents that for the 14-gage in a 345° F. oven. The correct baking time for the light metal in this oven has been determined to be about 9 minutes. At the end of 9 minutes the heavier sheet, however, has reached only 285° F., and if it is removed from the oven at this time the enamel film is found to be soft, and to have poor water resistance and

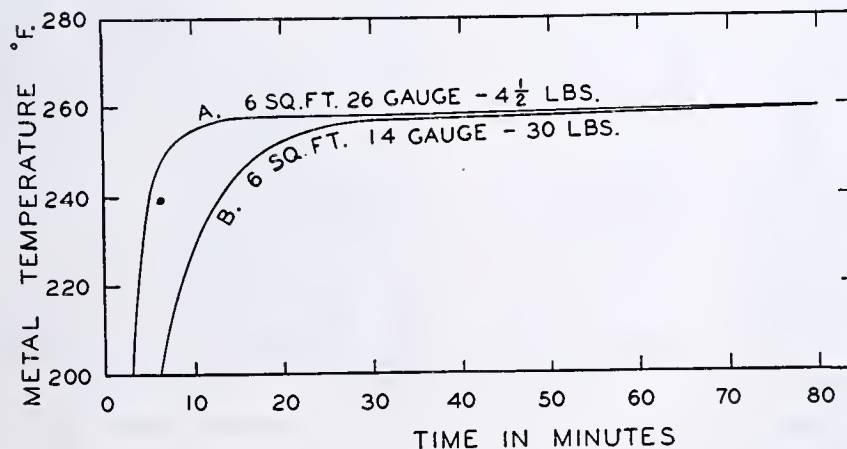


Figure 1. Temperature-Time Relationship



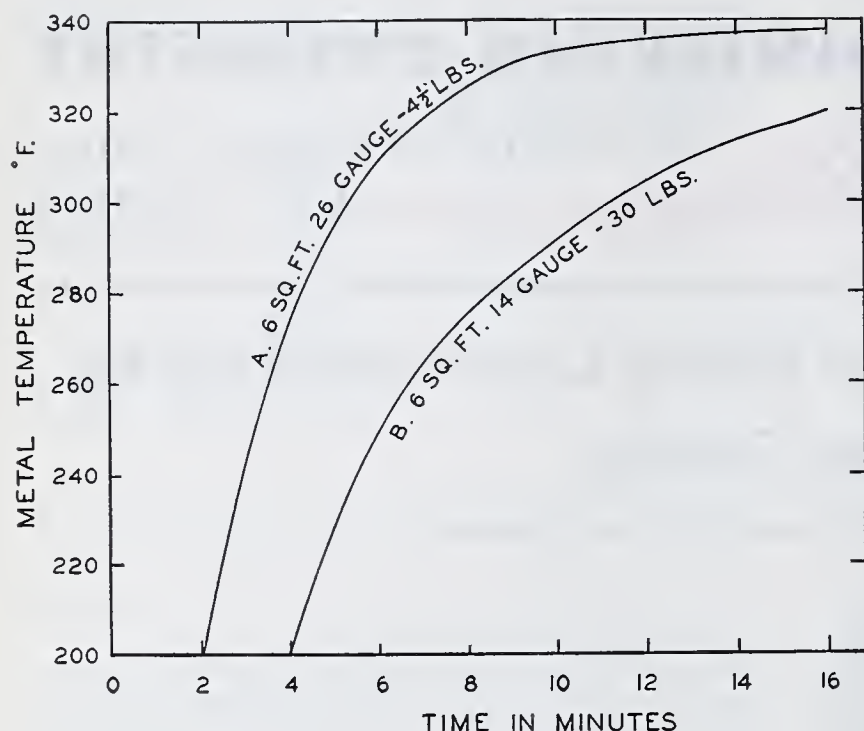


Figure 2. Time-Temperature Relationship

poor durability. As a matter of fact, the 30 pounds of heavy sheet must be baked about 16 minutes, or nearly twice as long, to obtain standard properties. The paint film on a 30-pound metal casting, of small surface area, is still tacky after the full 16 minutes in this oven. It must be baked approximately 40 minutes or nearly four times as long as the 26-gage metal to obtain usable film properties.

It is obvious that ware of varying thicknesses cannot be baked at the same high-temperature short-time schedule without encountering underbaking of the heavy pieces or overbaking with consequent poor color and brittleness of the light-weight pieces. This has been demonstrated in connection with numerous industrial applications where the operators have established the correct short baking schedule for sheet metal ware, only to find that heavy gear housings or other cast parts baked at the same time were so badly underbaked that gasoline used to remove grease after the subsequent assembling operation would wash off the finish.

There are also factors other than the mass of the metal which affect the baking speed of the finish—for instance, dark-colored bonderited metal sheets heat up much more rapidly than bright steel because of the heat reflectance from the backs of the latter. Enamels of varying colors have different heating rates for the same reasons. Various ovens heat a charge of ware at varying rates because of their varying heat capacities and rates of air circulation. In box-type ovens, the length of time during which the door is open for charging the oven has a very noticeable effect. These variables affect baking operations in all temperature ranges, but have a much greater effect on high-temperature bakes.

#### DETERMINATION OF BAKING VALUES

A method for determining the progress of an individual baking operation which will take into account the ac-

celeration or deceleration of the bake caused by these various external influences is badly needed.

Since the curing or heat polymerization of an organic enamel film is essentially a chemical reaction, it should be possible to determine the relationship between temperature and velocity for this reaction, and by means of metal or paint film temperature determinations to calculate the progress of the polymerization reaction and determine the time at which it is completed.

The total baking effect on paint film is the summation of the individual baking effects obtained during short increments of time, such as one minute, the temperature during these increments varying from room temperature up to a maximum approaching or equaling the air temperature of the oven. It has been demonstrated that for the particular urea-formaldehyde type of industrial enamel with which this work was conducted, the reaction velocity at temperatures below 240° F. is so low that a minute of time below this temperature has a negligible baking effect, although the threshold temperature for other types of finishes might obviously be higher or lower. In Figure 3, the total bake on the ware is the sum of the baking effect represented by *a* and that represented by *b*, *c*, *d*, etc., each increment of time being, for the purpose of illustration, one minute.

The problem is now to determine the baking value of these various increments or, in other words, to determine the relationship between the reaction velocity and temperature for the particular product under consideration. Assuming that the reaction velocity *vs.* temperature curve for the polymerization reaction, like that for other chemical reactions, is logarithmic in nature, it should be possible to determine the reaction velocity at two or three relatively low temperatures where the baking time is long in comparison with the time required to bring the ware up to the temperature of the oven, and to extrapolate with reasonable accuracy to higher temperatures. At these low temperatures only slight errors are introduced by the necessity of estimating the effective starting time of the baking operation at the temperature under investigation. At the higher temperatures, the reaction may be completed before the test film reaches the temperature being studied.

Paint films have been applied to very thin sheet metal, which comes up to the oven temperature in a relatively short

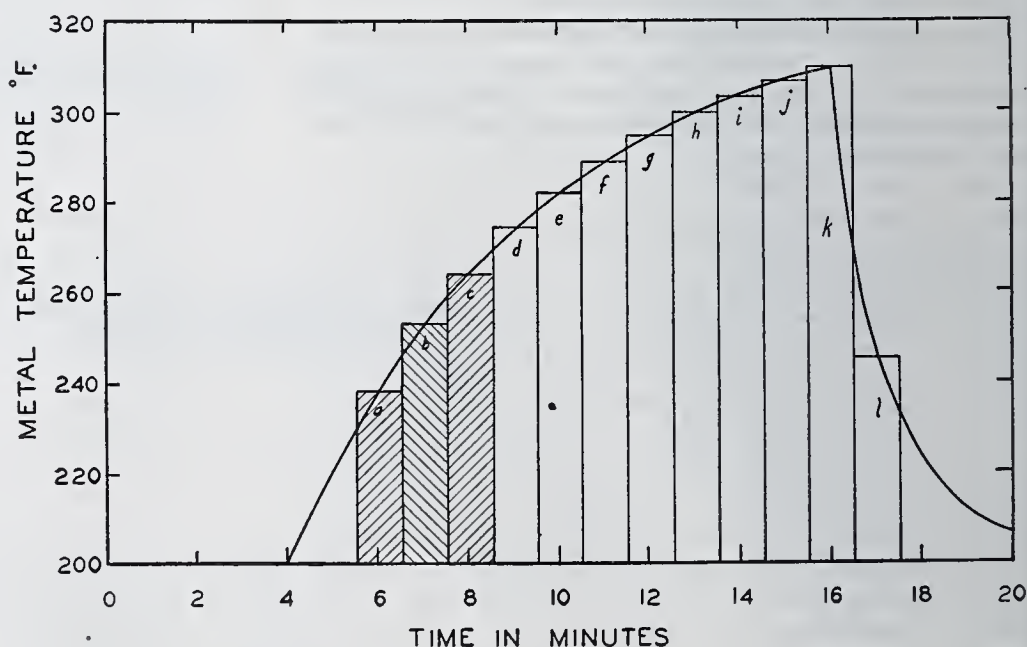


Figure 3



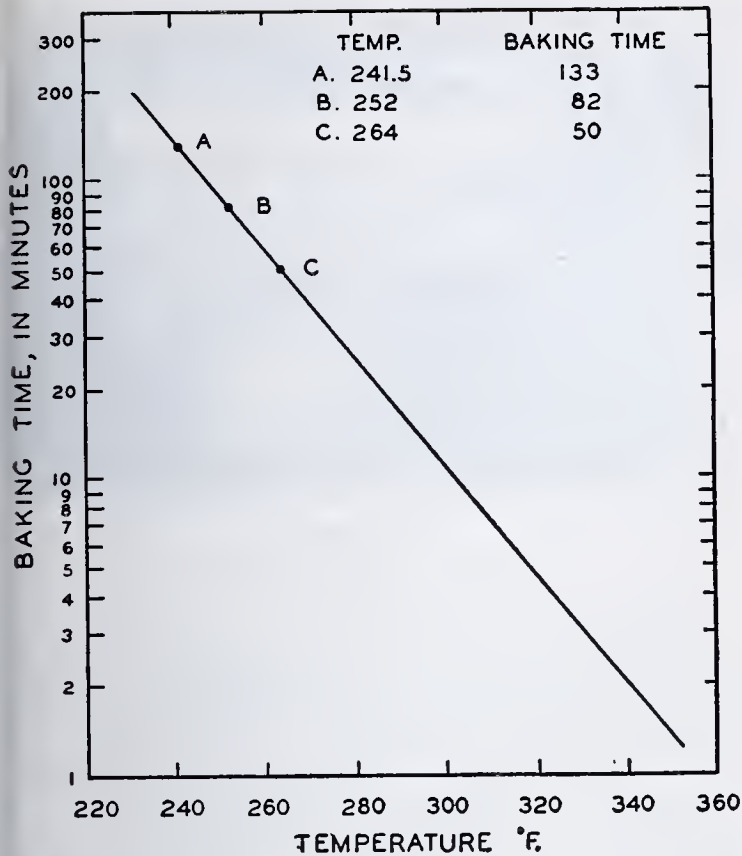


Figure 4. Time-Temperature Curve

time, and baked at 241.5°, 252°, and 264° F. until the arbitrarily selected standard film properties were obtained. By examining the films for these properties, the correct baking times for these temperatures have been determined to be, respectively, 133, 82, and 50 minutes. In Figure 4 these values were plotted on a logarithmic scale against temperature. As predicted, they lie roughly in a straight line, the error being well within the limit of error in the method of determining the reaction end point. The extension of this line represents the extrapolation of these points to higher temperatures.

For any given temperature the reciprocal of the baking time, or time in minutes required to give a complete bake, multiplied by 100, gives the per cent of bake acquired in one minute at that temperature. Figure 5 represents per cent bake per minute *vs.* temperature as converted from the baking time *vs.* temperature curve in Figure 4. It is plotted on a linear scale.

The curve was tested by using it as a guide, as described below, in making bakes at various temperatures from 240° up to 340° F. At all temperatures comparable bakes were obtained as judged by color, gloss, and other properties regardless of the oven loading, thickness of the metal, etc. The original assumptions concerning the relationship between baking speed and temperature, and the logarithmic shape of the reaction velocity *vs.* temperature curve are shown to be correct. Occasionally, when curves of this type are being calculated the expected errors of extrapolation are encountered, resulting in consistent over- or underbakes in the high-temperature range. In these cases the slope of the logarithmic curve must be re-established by trial and error methods.

The curve in Figure 5, then, provides a means for integrating the time-temperature curve for a baking operation or, in other words, for summing up the various baking values for the 1-minute increments as the bake proceeds. The metal temperature is determined every minute, converted by means of Figure 5 to per cent bake per minute, and added to the previous value. When a total of 100% is reached the ware is removed from the oven.

An examination of Figure 5 reveals many of the reasons for the

difficulties previously encountered in controlling high-temperature, short-time baking operations. In the range of 340° (metal temperature) the paint film is baking at the rate of 50% per minute. In other words, a 1-minute variation in the time at which the ware is removed from the oven gives a 50% error in effective bake. In this temperature range a slump of 10° for only 1 minute, due to power failure, opening of the oven doors, etc., will result in a 15 to 20% decrease in effective bake. Because of the number of variables which affect baking operations, it is common to find variations of as much as 20° to 30° in metal temperature, during the heating up period, from one bake to the next, seemingly conducted under the same conditions. Figure 5

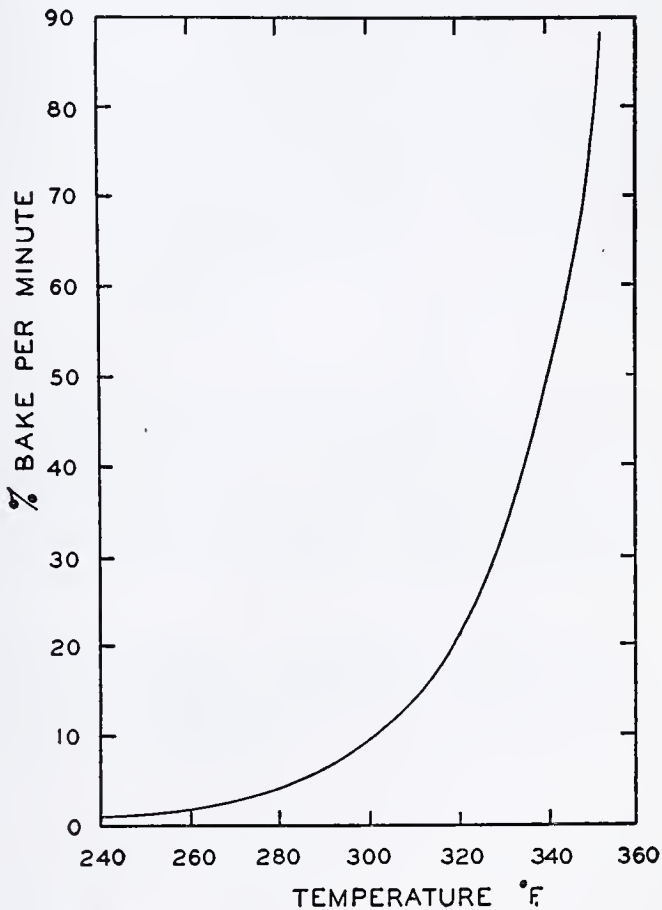


Figure 5. Integration of Time-Temperature Curve

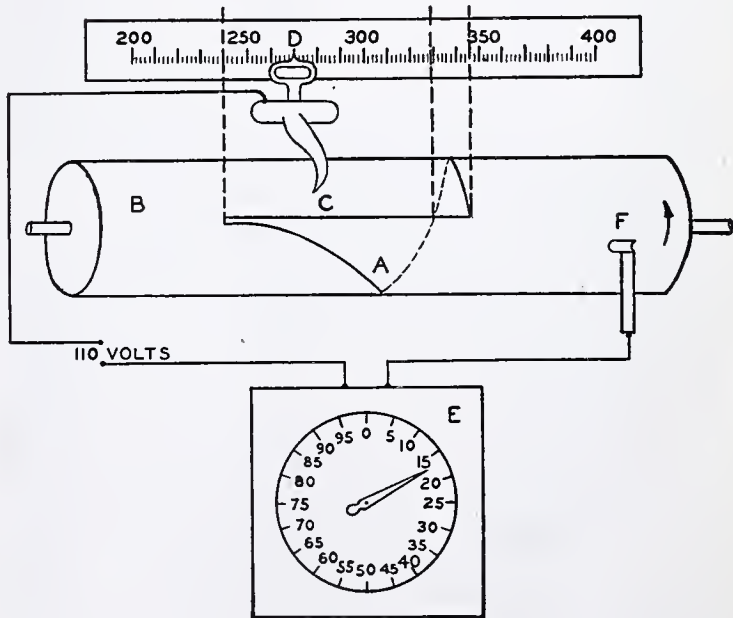


Figure 6. Diagram of Instrument



demonstrates the serious error in effective bake which will result from these variations. Attempts to control bakes by determining air temperature result in large errors because the metal temperature lags considerably behind the air temperature and experience has shown that the magnitude of this lag is entirely unpredictable, especially when oven charges of various weights per unit area are being handled.

The method of controlling baking operations as described has proved to be foolproof and has resulted in consistently equivalent film properties on various types of ware from thin sheet metal to heavy castings baked under widely varying conditions. However, precautions must still be taken against baking pieces of varying weights per unit area or varying colors during the same operation.

Most of this work has been confined to a single type of urea-formaldehyde industrial enamel plasticized with a nondrying oil alkyd resin. There is evidence available to show that the same curve can also be used with some finishes of other types, including other urea-formaldehyde resins, melamine resins, phenolics, etc., by merely shifting the curve vertically. However, it appears that for certain types of finishes, such as straight alkyds, a shift in the slope of the curve might be necessary.

The method could very possibly be adapted to the control of other chemical reactions such as heat bodying of oils.

#### AUTOMATIC INSTRUMENT

The method has the obvious disadvantage that it requires the constant attention of an operator who must determine the temperature of the metal once every minute or at other suitable short intervals, convert to per cent bake by means of the curve or a table, and add the results. To overcome this disadvantage, an instrument has been designed which does this work automatically.

The basis of the instrument is a Brown recording potentiometer pyrometer, which determines the temperature of the ware by means of a contact thermocouple and indicates the temperature on a horizontal linear scale. In its original form the instrument also traces a time *vs.* temperature curve on a sheet of paper moving at constant speed over a revolving drum. In its revised form the chart drum has been replaced by another drum (B, Figure 6) which revolves at a constant speed, preferably about 3 r.p.m., although the exact speed is immaterial. The surface of the drum is replaced by a covering of electrically conducting material, A, the shape of which is equal to that of the temperature *vs.* per cent bake per minute curve (Figure 5). The temperature axis of the curve is drawn to the same scale as the horizontal temperature scale of the potentiometer. The per cent bake per minute axis is drawn to such a scale that, at that temperature at which the baking rate is 60% per minute (in this case 345° F.), it equals the circumference of the cylinder, as is explained below. The remainder of the surface of the cylinder is constructed of non-conducting material. The original pen of the instrument is replaced by a contact pointer, C. An electric seconds timer is connected in such a manner that the electric circuit which operates the timer is completed when pointer C is in contact with the conducting cylinder covering A through the brush, F.

When a baking operation is started, pointer C gradually moves to the right as the temperature of the metal ware in the oven rises. When the minimum effective baking temperature, in this case 240°, is reached, the pointer is in contact with the conductor covering A during a part of the revolution of the cylinder, during which time the pointer of counter E moves a fraction of a unit. At this temperature the width of the conducting covering by determination from the curve in Figure 5 is such that during every minute of operation the contact pointer engages the conducting covering for a total of 0.773 second; hence the timer pointer moves 0.773 second. This value of 0.773 is equal to the per cent bake acquired by the finish during 1 minute at this temperature. As the temperature rises pointer C periodically measures off the height of the conducting segment of the cylinder and the pointer of counter E adds the resulting value to that summed up previously. It is seen by examination of the diagram that at higher temperature the conducting covering is wider and the timer pointer moves a constantly increasing distance for each revolution of the cylinder.

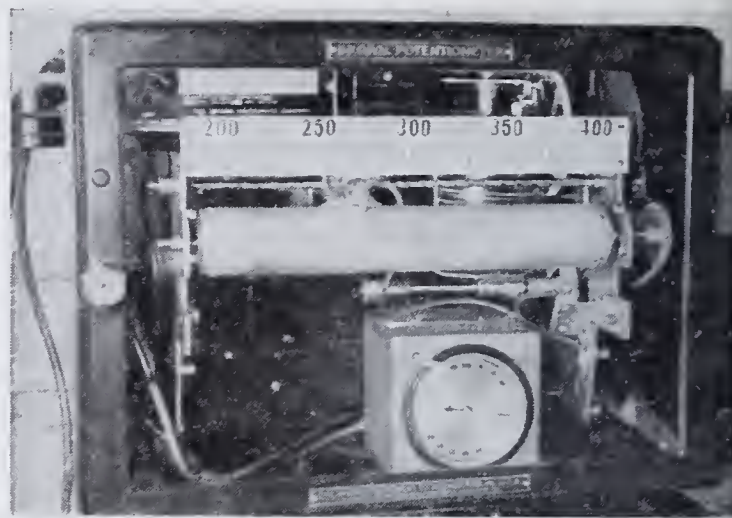


Figure 7. Automatic Instrument

At 328° F., for instance, the width of the conducting covering is equal to one half of the circumference of the cylinder and the circuit is closed for one half of the time during each revolution. In other words, the pointer adds up 30 seconds or units during each minute of bake, and the finish is baking at the rate of 30% per minute. The pointer of counter E, therefore, reads directly in per cent bake. Likewise, at 345° F. the contact pointer engages the conducting covering during the entire revolution of the cylinder and the timer pointer moves continuously; the finish is baking at the rate of 60% per minute. If at any time during the bake the oven door is opened or the power is shut off so that the temperature of the metal drops off, the instrument will follow this dip in temperature, since the number of units which the counter pointer moves per unit of time will also decrease.

As the bake proceeds, it is only necessary to glance periodically at the pointer. As it approaches 100% the ware is removed from the oven.

In the case of another type of finish with a faster baking speed but whose per cent bake per minute *vs.* temperature curve has the same slope, it is necessary only to apply a predetermined factor, baking, for instance, to 75% instead of 100%. If the curve has a different slope, it is necessary to construct an interchangeable cylinder for the instrument. Cylinders can also be constructed to cover a wider range of baking temperatures, in which case it is necessary to make suitable alterations in the scale of the electric timer to agree with the revised ordinate of the conducting curve on the cylinder.

A photograph of the instrument is shown in Figure 7.

## Volume Correction Factors for C<sub>4</sub> Hydrocarbon Mixtures

National Bureau of Standards Letter Circular LC-757, compiled by C. S. Cragoe, presents in 23 pages volume correction factors for C<sub>4</sub> hydrocarbon mixtures compiled at the request of the Rubber Reserve Co. to supplement tables on pure hydrocarbons issued November 23, 1943, to supply standard tables especially applicable to C<sub>4</sub> mixtures covering a wide range of composition, to facilitate accurate determinations of quantities bought and sold in commercial transactions, and to supersede other less accurate tables. One of its major objectives is standardization of methods of correcting volumes observed at various temperatures to the conventional standard units (gallons at 60° F.) in commercial transactions involving C<sub>4</sub> hydrocarbon mixtures, particularly those used in the manufacture of aviation gasoline and butadiene. Copies are available on request from the National Bureau of Standards, Washington 25, D. C.



# Detection of Destructively Distilled Wood Turpentine In Other Kinds of Turpentine by Means of the Aniline Point

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The presence of destructively distilled wood turpentine in gum spirits, and perhaps also in steam-distilled wood turpentine, may be detected by aniline point tests on the high-boiling fraction distilling above 170° C., described in this paper.

Four kinds of turpentine are recognized under the Federal Naval Stores Act—gum spirits of turpentine, steam-distilled wood turpentine, sulfate wood turpentine, and destructively distilled wood turpentine. The first three are produced by processes in which the oleoresin from which the terpene constituents are derived is subjected to relatively low temperatures (in the presence of a large amount of water vapor), whereas in the destructive distillation process much higher temperatures are used to effect the dry decomposition of the wood. These high temperatures in the dry distillation process result in the formation of various complex hydrocarbons and oils, some of which are closely related to the aromatic or benzene hydrocarbons, and some are perhaps of unknown identity.

Destructively distilled wood turpentine usually sells at a price below the established market price for gum spirits of turpentine or steam-distilled wood turpentine. The price differential has at times induced unscrupulous dealers to adulterate gum spirits and steam-distilled wood turpentine by adding small quantities of the destructively distilled wood turpentine. The sale of mixtures of this kind in interstate commerce is injurious to commerce in naval stores and prejudicial to the sale of pure turpentine, and is therefore prohibited by the Federal Naval Stores Act. The wholesale price of sulfate wood turpentine is also usually below that of the other two kinds; consequently, its adulteration could hardly be economically feasible.

This type of adulteration can usually be detected, especially by a person experienced in the testing of turpentine, because of the characteristic odor of the adulterant. However, to provide legally acceptable evidence, the analyst needs some method of evaluation based on scientific fact or recordable data—entirely independent of the personal element based on a sensory observation—on which to support his findings.

As indicated by the standard specifications under which the several kinds of turpentine are produced and sold, destructively distilled wood turpentine contains appreciable quantities of constituents distilling in the range from 170° to 180° C. (1, 4), whereas in gum spirits and steam-distilled turpentine (1, 3) which consist chiefly of  $\alpha$ - and  $\beta$ -pinene, these higher boiling constituents are present in only relatively small quantity.

Another difference between destructively distilled and other types of turpentine owing to the difference in composition, is the greater so-called "solvent power" of the former. The two most commonly used methods for evaluating the solvency of paint thinners are the kauri-butanol (5) and the aniline point tests (6). The kauri-butanol test is subject to wide variation due to difficulty in temperature control, and each new solution of kauri gum must be standardized to establish reference points. The aniline point test, on the other hand, is relatively simple, is the only commonly used solvency test in which close temperature control is not a factor, and is widely used to evaluate paint thinners and solvents. (The aniline point of a diluent or solvent is the minimum equilibrium solution temperature for equal volumes of ani-

line and the solvent.) It was, therefore, considered by the authors that these two differences in properties might serve as a means of proving the presence of destructively distilled wood turpentine in other kinds of turpentine. No reliable method of detection based on chemical reactions or phenomena has as yet been found.

A preliminary study of the aniline points of authentic samples of the various turpentines from widely separated sources gave the following results: gum spirits (24 samples), 12.2° to 14.5° C.; steam-distilled turpentine (11 samples), 19° to 25.5° C.; sulfate wood turpentine (2 samples), 15° and 18° C.; destructively distilled turpentine (4 samples), all below -10° C.

In the initial stage of this study, six samples of pure gum turpentines were fractionated and aliquots collected on a volumetric basis, without reference to the distilling temperature. Similar fractionations were made on these turpentines containing 5 and 10% of added destructively distilled wood turpentine. The aniline points of the various fractions from the adulterated turpentine were not sufficiently different (lower) from those of the pure turpentine fractions to permit any definite conclusions.

After several preliminary tests, fractionations were made on single 1-liter samples of a pure gum spirits, a steam-distilled, a sulfate, and a destructively distilled wood turpentine, using a 250-mm. Vigreux fractionating column. The fractions were collected as follows: below 160° C.; from 160° to 163° C.; 163° to 167° C.; 167° to 170° C.; and all distilling above 170° C. (With smaller samples a 150-mm. column would be more suitable.) The aniline points of the fractions obtained by this type of separation showed that there was enough difference between destructively distilled turpentine and the other kinds to suggest that this test might serve as the basis for a method for positive identification or proof of its presence in a suspected mixture.

Table I. Mixed Aniline Points of Fractions of Destructively Distilled Wood Turpentine, Collected Above 170° C.

Mineral Spirits (60° C., A.P.) %	Sample Number			
	1 ° C.	2 ° C.	3 ° C.	4 ° C.
0	< -10.0	< -10.0	< -10.0	< -10.0
10	- 6.0	-10.5	- 3.0	- 7.0
20	2.0	1.5	9.5	3.0
30	11.5	9.5	16.0	12.5
40	18.5	18.5	23.0	20.0
50	25.5	26.5	30.0	26.5
Yield of distillate over 170° C., %	18.4	16.0	50.3	25.2

Four authentic samples of destructively distilled wood turpentine from different producers were next fractionated through the column. The fractions collected above 170° C. were subjected to a series of aniline point tests, both on the fraction as collected and after mixing with varying proportions of a standard mineral spirits having an aniline point of 60° C. The similarity of the aniline points for these four samples is shown in Table I.

The next step in the study was the comparison of the high-boiling fractions, when recovered by distillation at atmospheric pressure, and when obtained at reduced pressure. Sample 1 was considered characteristic and was selected. It was first fractionated at atmospheric pressure throughout the distillation, the fraction above 170° C. being reserved. A similar distillation was made at atmospheric pressure up to 170° C., after which the higher fraction was collected at reduced pressure (40 mm.).

Five per cent by volume of this same turpentine was added to a quantity of one of the pure gum turpentines, and two similar fractionations were made on this adulterated sample. The four high-boiling fractions were subjected to a series of mixed aniline point tests (Table II). The results showed that even with as



little as 5% of destructively distilled wood turpentine in gum spirits a portion remains undistilled at 170° C. under atmospheric pressure; also that further distillation of this residue, either at atmospheric or preferably at reduced pressure, yields a fraction which has mixed aniline points that are in good agreement with those obtained on the similar fraction from the straight destructively distilled wood turpentine.

For the last series of tests, one authentic gum spirits and three authentic destructively distilled wood turpentines were selected. All four samples were first subjected to fractional distillation at atmospheric pressure. A series of mixed samples was prepared, each to contain destructively distilled turpentine in the gum turpentine, in the ratios of 1 to 19 (5%) and 1 to 9 (10%), and fractionated in the same manner. The straight aniline points of these fractions were determined (Table III).

Table II. Mixed Aniline Points

(Fractions of D.D. wood turpentine collected above 170° C. and of gum turpentine containing 5% of same D.D. wood turpentine)

Mineral Spirits (60° C., A.P.)	D.D. Wood Turpentine Straight distillation ° C.	D.D. Wood Turpentine Vacuum distillation <sup>a</sup> ° C.	95% Gum-5% D.D. Straight distillation ° C.	95% Gum-5% D.D. Vacuum distillation <sup>a</sup> ° C.
0	< -10.0	< -10.0	< -10.0	< -10.0
20	0.5	-3.0	2.5	-5.0
40	18.5	16.5	20.5	14.0
60	34.5	33.5	35.5	32.0
80	48.0	48.0	48.5	47.0
Distillate, %	28.0	30.8	2.1	5.0

<sup>a</sup> Material subjected to vacuum distillation was that remaining after atmospheric distilling temperature reached 170° C.

From the data obtained it is concluded that any opinion as to the presence of destructively distilled wood turpentine in gum spirits of turpentine, based on an olfactory detection of the characteristic pungent odor of the former, may be substantiated by aniline point tests on the higher boiling fractions. The odor of the adulterant, when present in only small quantity, was more easily recognized in the higher fractions.

Since the straight aniline points of the samples of gum spirits of turpentine were closer to those of the destructively distilled wood turpentines than was the case with the steam-distilled wood turpentines initially tested, it was felt that any conclusions or procedure based on a depression of aniline point of fractions from a gum spirits would hold for most steam-distilled wood turpentines. No comparable series of tests was therefore made with this latter class.

Steam-distilled wood turpentine, which is produced by a rather complicated refining process from the original distillate as it comes from the wood, may be poorly refined and contain small quantities of dipentene or pine oil which might also serve to depress the aniline points of the higher fractions. However, these substances are usually removed by the manufacturer to the greatest extent possible, since they have become more valuable than the turpentine. They must be removed if the turpentine is to meet the standard specifications for this class, which are identical with the specifications for gum spirits. The chances of drawing false conclusions from the aniline point data obtained by means of the procedure herein described are believed to be remote enough to warrant the use of the method also on samples of steam-distilled wood turpentine suspected of this type of adulteration, especially when the presence of the destructively distilled wood turpentine is suggested, even though faintly, by the odor.

The small quantities of material on which the aniline point tests had to be run, and the low temperatures obtained, made it necessary to develop a test assembly different from the apparatus used in the standard A.S.T.M. aniline point determination (2). The apparatus is shown in Figure 1.

Test tube B is supported by a heavy cardboard cover over C, which at the same time serves to insulate the surface of the liquid therein from the surrounding warmer atmosphere.

For most purposes, the following procedure should give satisfactory and reliable results. Fractionate from 250 ml. to 1 liter

of the sample through an efficient distilling column, at atmospheric pressure, carrying the temperature of the distilling vapor up to 170° C. The distillate up to this point is not used. Continue the fractionation, if possible, under slight vacuum, until the distillation stops or decomposition begins. Dry this fraction by shaking with anhydrous sodium carbonate, and filtering, preferably through a fritted glass-bottom crucible of medium porosity. Pipet 3 ml. of the dried sample into test tube A and add 3 ml. of pure fresh aniline. (Only pure, fresh aniline will serve the purpose. Old aniline must be purified by redistilling after treatment with anhydrous sodium carbonate, rejecting the first and last 10% of distillate. As aniline is toxic, even through the skin; it should be handled with caution and should never be directly pipetted by placing the end of the pipet itself in the mouth.)

Fill baths B and C with prechilled alcohol or acetone, the temperature of which is gradually reduced by the addition, at intervals, of small pieces of dry ice. However, if this is not available it is possible to obtain a temperature of -21° C. by using a mixture of equal parts of 66% sulfuric acid, precooled to 0° C., and finely crushed ice. Immerse the tube in the chilling or low-temperature bath, B, until the solution in A clouds. Remove the tube and agitate the contents with the copper wire in a shuttle-like manner during the observation of the aniline point. Record the temperature at the instant the solution clears. Repeat the operation until duplicate operations or readings agree within  $\pm 0.2^\circ \text{C}$ .

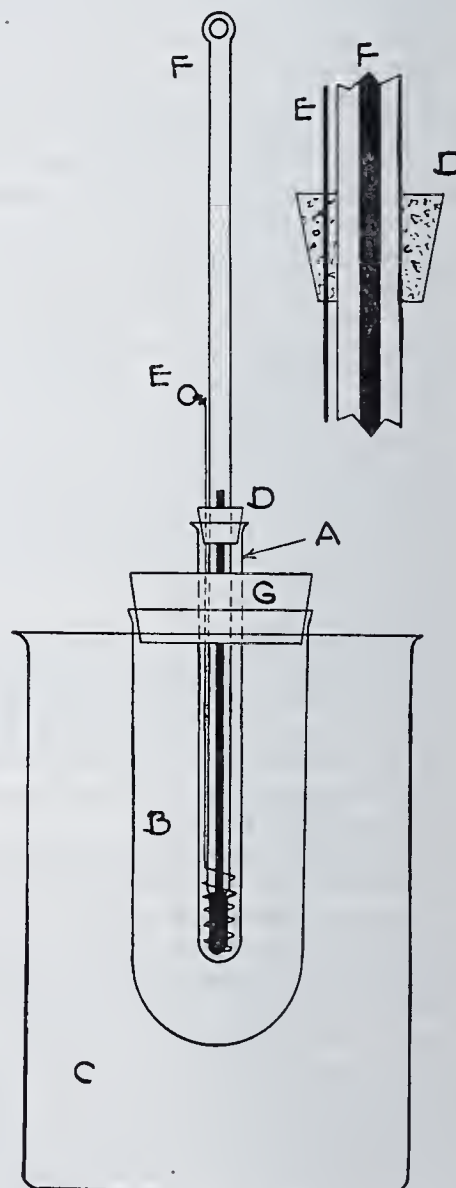


Figure 1. Assembly for Determining Aniline Points

- A. 15-ml. test tube, with lip, 12.5 × 1.25 cm. (5 × 0.5 inches)
- B. 200-ml. test tube, with lip 12.5 × 5 cm. (5 × 2 inches)
- C. 100-ml. beaker
- D. Cork stopper, to exclude moisture
- E. Copper wire for agitation, coiled at base
- F. Low aniline point thermometer -38 to +42° C., A.S.T.M., E-1 (33C-41-T)
- G. Cork stopper



**CONCLUSIONS**

Recordable data to prove the presence of destructively distilled wood turpentine in gum spirits, and perhaps in steam-distilled wood turpentine, may be had by subjecting the high-boiling fraction distilling above 160° C. to an aniline point determination. The presence of such adulteration, particularly if so indicated by odor, would be verified: (1) if the straight aniline point of the whole sample is lower than +12° C.

- (2) if a distillation fraction, using a column, is obtained which stills above 170° C.
- (3) if the straight aniline point of this fraction is lower than 10° C.
- (4) if the mixed aniline point of such fraction, using a 50-50 mixture of the fraction and a standard 60° C. A.P. mineral spirits, is lower than +30° C.

Table III. Aniline Points of Fractional Distillates of Gum Spirits and Destructively Distilled Wood Turpentine and Mixtures Thereof

	Original Sample				Mixed Sample Gum with					
	Gum ° C.	D.D. No. 1 ° C.	D.D. No. 2 ° C.	D.D. No. 3 ° C.	5% No. 1 ° C.	10% No. 1 ° C.	5% No. 2 ° C.	10% No. 2 ° C.	5% No. 3 ° C.	10% No. 3 ° C.
Whole Sample	12.2	-20.4	-20.0	-20.8	10.5	9.1	10.4	9.0	10.2	8.8
Fraction distilling										
Below 160° C.	17.6	-24.2	-23.0	..	15.7	14.8	16.1	14.3	17.3	16.3
160-163° C.	13.4	-22.0	-19.0	..	11.5	11.9	12.4	11.4	13.1	12.0
163-167° C.	3.8	-13.0	-15.0	- 9.0	2.7	3.1	3.5	2.1	5.1	7.0
167-170° C.	-10.6	-15.0	-17.0	- 9.0	- 9.0	- 8.0	-10.4	- 9.0	- 5.0	- 3.5
Above 170° C.	..	-22.0	-21.4	-18.6	<-30.0	-16.8	-22.0	<-30.0	-15.0	-14.4

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# Physical Constants of Methyl Esters of Commonly Occurring Fatty Acids

## VAPOR PRESSURE

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Methyl esters of caproic, caprylic, capric, lauric, myristic, palmitic, stearic, oleic, and linolic acids were obtained in a pure state by repeated fractional distillation. Vapor pressure curves and decomposition pressures and temperatures have been determined for each of the methyl esters by the method described by Ramsay and Young. With the exception of the C<sub>18</sub> series, it has been shown that an ester can be identified and its purity ascertained by means of its vapor pressure curve. With the aid of the decomposition data, it is possible to eliminate excess decomposition by controlling the pressure and hence the boiling temperature of fractional distillation.

Fractional distillation is recognized as an excellent method for separating and purifying the methyl esters of the naturally occurring fatty acids. The fractions obtained from such a distillation may be divided into two classes: the pure ester fractions, and the mixed fractions containing two or more esters. Two types of analyses are possible for the identification of such fractions. The best and most commonly used are chemical analyses which involve the determination of such values as the iodine number, neutral equivalent, and thiocyanogen number. These procedures are difficult and time-consuming, and are impractical when the fractions are very small. Accordingly, it seemed advisable to investigate the second possibility, that of substituting physical measurements for the usual chemical methods. Consequently, the problem resolved itself into a search for suitable physical constants which would yield information as to the purity and quantity of any ester in a given fraction.

Vapor pressure was the first physical constant investigated. By means of this determination the purity of a substance may be ascertained in a very short time and with a considerable degree of accuracy. By choosing a dynamic method, it is also possible to

find the boiling point of the substance in question at any given pressure. This last fact in itself is of great importance in the separation and purification of materials by fractional distillation. It is also possible to learn from a vapor pressure determination the temperature and pressure at which decomposition occurs. This information can be utilized to great advantage in the fractional distillation of organic compounds.

PREPARATION OF THE METHYL ESTERS

The methyl esters of caproic, caprylic, capric, lauric, myristic, palmitic, stearic, and oleic acids were prepared and purified by repeated fractional distillation through a 16-plate, electrically heated fractionating column, of the type described by Whitmore and Lux (2), fitted with a total reflux, partial take-off distilling head. The purity of each ester was determined by the usual chemical analyses, consisting of neutral equivalent, iodine number, and thiocyanogen number. The methyl ester of linolic acid was purified first, by repeated crystallization of the tetrabromo derivative, and then, after regeneration, by repeated fractional distillations. These pure esters were used for the entire study of the physical constants.

DETERMINATION OF VAPOR PRESSURE

The vapor pressure curve for each methyl ester was determined by the dynamic method described by Ramsay and Young (1); which was found to give very accurate results for all esters, including those which are normally solid at room temperature. This method was chosen because it requires a very small amount of material (approximately 1 ml.) which can be recovered, providing no decomposition takes place during the determination.

In Figures 1 and 2 are shown the vapor pressure curves for the pure methyl esters of the more commonly occurring fatty acids. The straight-line curves, drawn on semilog paper, were obtained by plotting the reciprocal of the absolute temperature  $\times 1000$  against the log of the pressure in millimeters of mercury. The curves, thus constructed, were extrapolated to 760-mm. pressure,



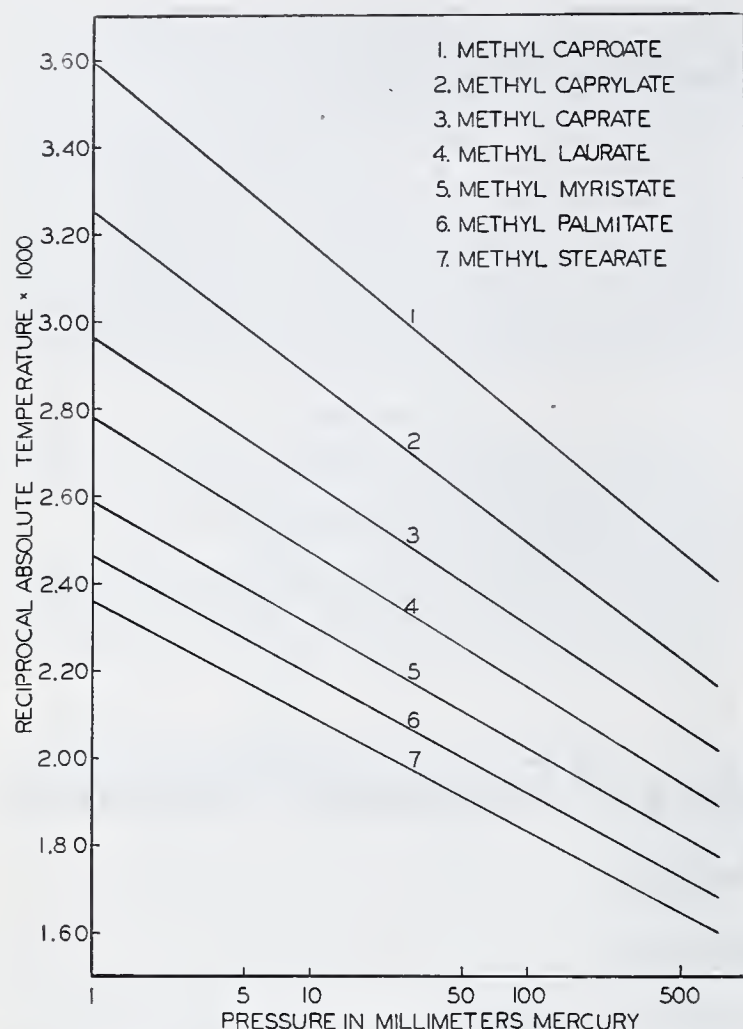


Figure 1. Vapor Pressure Curves of Methyl Esters of More Commonly Occurring Fatty Acids

since in the majority of the cases decomposition occurs far below that pressure. Table I gives the approximate decomposition pressures and temperatures for each of the methyl esters studied. Table II shows the boiling points for each of the esters at several different pressures.

#### CONCLUSIONS

From the data collected, several significant conclusions may be drawn.

With the exception of the  $C_{18}$  series, whose vapor pressure curves as shown by Figure 2 are practically identical, it is possible to identify a pure ester fraction by means of its vapor pressure curve. With the above information available, it is an easy matter to find the boiling point of an unknown ester at several different pressures, and then by superimposing the curve thus constructed on one of the above standard curves, to identify the ester. Such identifications can be accomplished in a very short time and with a considerable degree of accuracy. The purity of a known ester may be determined in the same way. The ester is pure if the constructed curve can be superimposed on the standard vapor pressure curve of the ester in question. Any slight deviation from purity will be recognized immediately, inasmuch as the two curves will not coincide.

Table I. Decomposition Pressures and Temperatures of Methyl Esters of Fatty Acids

Ester	Pressure, Mm. Hg	Temperature, ° C.
Methyl caproate	>760	>150
Methyl caprylate	>760	>193
Methyl caprate	>760	>224
Methyl laurate	160	204
Methyl myristate	60	205
Methyl palmitate	25	151
Methyl stearate	18	221
Methyl oleate	16	217
Methyl linolate	11	208

Table II. Boiling Points of Methyl Esters at Various Pressures

Ester	Pressure in Millimeters of Hg						
	2 ° C.	4 ° C.	6 ° C.	8 ° C.	10 ° C.	20 ° C.	40 ° C.
Methyl caproate	15	26	33	38	42	55	70
Methyl caprylate	45	58	65	71	76	89	106
Methyl caprate	77	89	97	103	108	123	139
Methyl laurate	100	113	121	128	134	149	166
Methyl myristate	127	141	150	157	162	177	197
Methyl palmitate	148	162	172	177	184	202	a
Methyl stearate	166	181	191	199	204	a	a
Methyl oleate	166.2	182	192	199.5	205.3	a	a
Methyl linolate	166.5	182.4	193	199.9	206	a	a

a Decomposes.

It is possible to adjust the pressure and hence the boiling temperature of a fractional distillation to optimum or nearly optimum conditions by making use of the recorded decomposition pressures and temperatures. The authors believe that during fractional distillation there is a tendency toward unnecessary superheating of the material to be distilled. This tendency can be eliminated through the use of decomposition data on the various esters.

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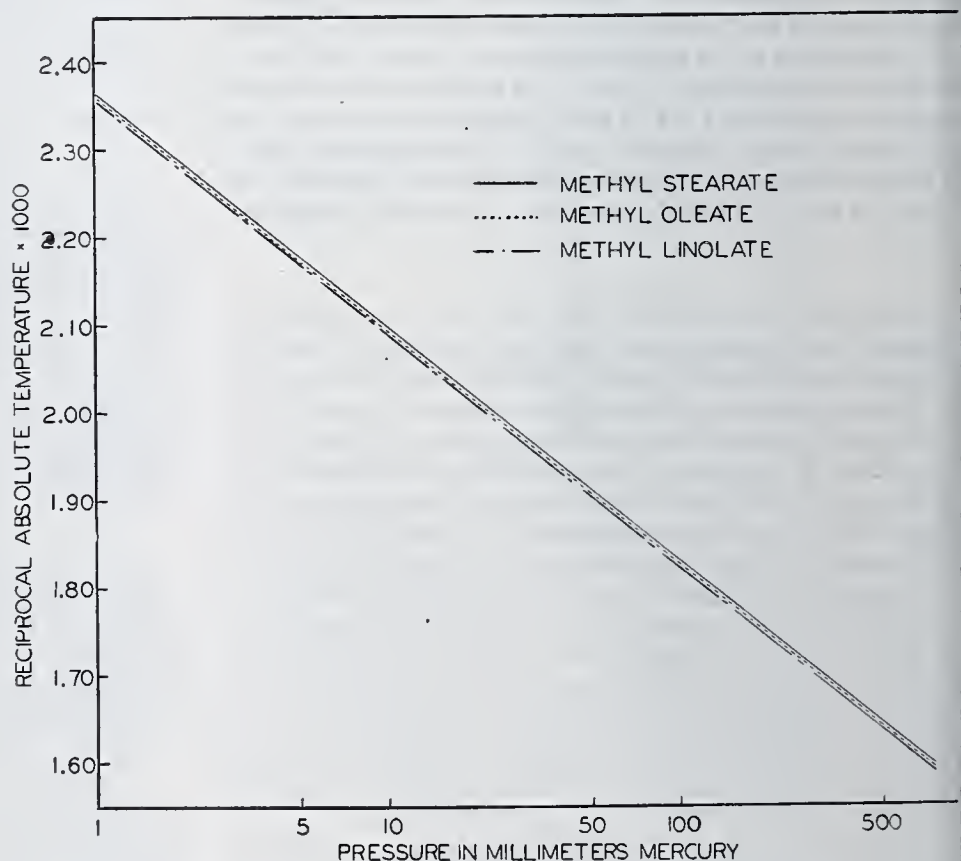


Figure 2. Vapor Pressure Curves of Methyl Esters of More Commonly Occurring Fatty Acids



# Spectrographic Determination of Small Amounts of Tungsten in Steel

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Accurate determinations of 0.01 to 0.25% of tungsten in steels were made by a combined spectrochemical method. The tungsten was separated by a variation of Knowles' method and then determined spectrographically. A modified chemical procedure for separating the columbium from tungsten was developed in order to determine tungsten spectrographically. A high degree of reproducibility is obtained by sparking carbon electrodes impregnated with the tungsten solution.

THE determination of small amounts of tungsten (0.01 to 0.25%) in steel has not been accomplished with satisfactory accuracy up to the present time. In the colorimetric determination of tungsten interference is caused by trace amounts of molybdenum (17-20). To date no successful spectrographic procedure has been reported which allows the determination of tungsten in the above-mentioned range, using solid steel specimens directly or simple solution procedure (3, 7, 10, 11, 14, 15). Thus a project combining both chemical and spectrographic methods was undertaken in determining residual tungsten in steel.

The tungsten is first separated chemically by a modification of Knowles' method (8, 18, 19) and all but a trace of molybdenum is removed by volatilization. The spectrographic procedure is then employed, using an alkaline solution of the tungstic oxide, with an aluminum salt added as an internal standard. A spark solution method (16) is employed which has a high degree of reproducibility, unobtainable in this case by the usual color method (2, 10, 12, 13, 14). The use of a medium spectrograph with its high optical efficiency and efficient resolving power was found to be satisfactory. The tungsten content can then be determined accurately by densitometric measurements.

## CHEMICAL PROCEDURE

Dissolve a 2-gram sample in a covered 250-ml. beaker with 10 ml. of hydrochloric acid (sp. gr. 1.19) and 30 ml. of perchloric acid (70%). Heat over a low flame until the sample is completely dissolved, then remove the cover glass, then add 1 to 2 ml. of hydrofluoric acid (48%). Increase the heat and evaporate to fumes of perchloric acid. When the chromium begins to oxidize, denoting the decomposition of the carbides, remove the beaker from the heat and allow it to cool until the salts begin to crystallize. Wash down the sides of the beaker with about 20 ml. of distilled water and swirl the salts into solution. Add 20 ml. of saturated sulfuric acid and heat until the sulfur dioxide is completely driven off. Cool to 10° C. in an ice bath, then add 3 ml. of ammonium molybdate solution (0.0054 gram of molybdenum per ml.) and some paper pulp and mix well. Add slowly 15 ml. of a 2% alcoholic solution of  $\alpha$ -benzoin oxime while stirring and a sufficient quantity of saturated bromine water to tint the solution orange. Add 15 ml. more of the  $\alpha$ -benzoin oxime solution and repeat the foregoing procedure. Allow to stand 10 minutes with occasional stirring, adding more bromine water if necessary. Filter through No. 40 11.0-cm. Whatman paper containing paper pulp without allowing the precipitate to run dry. Break up the precipitate with  $\alpha$ -benzoin oxime wash solution (10 ml. of sulfuric acid sp. gr. 1.84, 965 ml. of distilled water, cool to 10° C., and add 25 ml. of alcoholic  $\alpha$ -benzoin oxime solution, 2%). Rinse and swab the beaker, and wash the precipitate with 150 ml. of the wash solution, breaking up the precipitate. Char and ignite in a platinum crucible at 734° C. for 2 hours or until there is no further sign of molybdenum volatilizing. Add to the residue in the platinum crucible 5.0 ml. of a stock solution containing 490 ml. of sodium hydroxide (0.5 molar) and 10 ml. of aluminum sulfate octadecahydrate (10%). Stir with a glass rod to macerate any lumps in

the residue. Warm on a steam bath for a few minutes, then filter through a dry No. 40 Whatman 9.0-cm. paper.

**MODIFIED CHEMICAL PROCEDURE WHEN COLUMBIUM IS PRESENT.** Dissolve a 2-gram sample in a covered 250-ml. beaker with 10 ml. of hydrochloric acid (sp. gr. 1.19), 5 ml. of phosphoric acid (85%), and 30 ml. of perchloric acid (70%). Heat over a low flame until the sample is completely dissolved. Continue as in chemical procedure.

## SPECTROGRAPHIC PROCEDURE

The carbon electrodes used in the spectrographic procedure are prepared by heating graphitic rods 0.78 × 30 cm. ( $\frac{5}{16}$  × 12 inches), in a muffle at 510° C. for 1 hour. After cooling, the rods are cut into 5-cm. (2-inch) lengths on an alundum cutting wheel (16). Before using, the ends of the electrodes are polished with a fine file. One drop of the solution, prepared by the above chemical procedure, is placed on each of two flat top carbon electrodes, which are sparked immediately after absorption of the solution. A Bausch & Lomb medium quartz spectrograph is used with an uncontrolled, condensed spark source, rated at 13,500 volts. The optical stand is placed 45 cm. (18 inches) from the slit. An exposure of 60 seconds with no condensing lens or pre-spark is recorded on an Eastman 33 plate 10 × 25 cm., (4 × 10 inches). The plate is developed for 4 minutes in D-19 at 18° C. and fixed for 15 minutes, followed by the usual process of washing and drying.

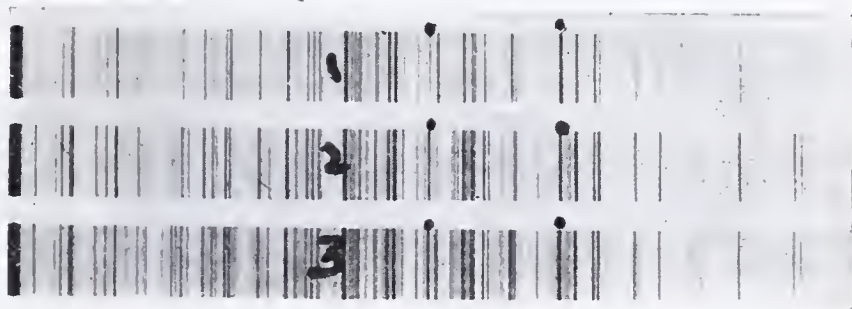


Figure 1. Spectra

1. 0.01% tungsten      2. 0.05% tungsten      3. 0.10% tungsten

The calibration of the plate is based on photometric measurement of lines having predetermined intensity values (1). The densities of the 2397.091 Å. tungsten line and the 2378.408 Å. aluminum reference line (4) are measured on a Leeds & Northrup recording microphotometer. The tungsten is then determined by the use of a working curve drawn by plotting  $\Delta \log I \frac{W}{Al}$  against percentage tungsten. Standard tungsten solutions are prepared for determining the values of the curve.

## PREPARATION OF STANDARDS

**STANDARD TUNGSTEN SOLUTION** (1 ml. = 0.001 gram of tungsten). One gram of pure tungsten metal is oxidized completely to yellow tungstic oxide in a muffle at 734° C. The tungstic oxide is then dissolved by warming in a solution containing 25 grams of sodium hydroxide in 100 ml. of distilled water. When solution is complete, it is transferred to a 1000-ml. volumetric flask and diluted to the mark.

A check of the standard solution is made by withdrawing 100 ml. of the solution and determining the tungsten by the cinchonine method (6).

## SPECTROGRAPHIC STANDARDS

To a corrosion-resistant steel (18-8) containing no tungsten, increments of the standard tungsten solution were added to give a



Table I. Reproducibility and Accuracy of Determinations<sup>a</sup>

Sample No.	Tungsten Added %	$\Delta \log I$	Maximum Deviation %
1	0.01	-56	0.002
2		-51	
3		-54	
4		-55	
5		-54	
1	0.03	-14	0.003
2		-15	
3		-15	
4		-18	
5		-18	
1	0.05	+04	0.006
2		+02	
3		+06	
4		+05	
5		+02	
1	0.07	+18	0.006
2		+19	
3		+15	
4		+16	
5		+17	
1	0.10	+32	0.003
2		+32	
3		+32	
4		+32	
5		+33	
1	0.12	+39	0.006
2		+39	
3		+40	
4		+39	
5		+41	

<sup>a</sup> Values for 0.13 to 0.25% tungsten determined from 1-gram samples by doubling results obtained.

range from 0.01 to 0.12% tungsten based on a 2-gram sample. For the range 0.13 to 0.25% tungsten, the same procedure was followed, based on a 1-gram sample. The tungsten separations were carried out according to the outlined chemical procedure. The spectrographic working curve was obtained from the values of these standards.

### DISCUSSION

The three spectra in Figure 1 show the gradation of the tungsten line 2397.091 Å., and the position of the aluminum reference line 2378.408 Å. The results in Tables I and II show the reproducibility and accuracy obtained for tungsten standards

Table II. Accuracy of Determinations

Bureau of Standards Sample	Type	Tungsten Present <sup>a</sup> %	Tungsten Found %	Deviation from Actual %
73a	Alloy, high Cr, low Ni	0.09	0.085	0.005
			0.087	0.003
			0.085	0.005
			0.085	0.005
123a	Corrosion-resistant steel, containing columbium	0.11	0.115	0.005
			0.103	0.007
			0.110	0.000
			0.108	0.002

<sup>a</sup> From Bureau of Standards certificate of analysis.

Bureau of Standard samples 73a and columbium-bearing corrosion-resistant steel 123a. The spectrographic standard solutions, as described, were used in the construction of the working curve. Pure tungsten solutions were not used, since it was considered desirable to approximate the conditions of routine analysis. Investigation showed that a working curve, prepared by using pure tungsten solutions, differed from one constructed by the above procedure. Although tungsten is claimed to be completely precipitated by  $\alpha$ -benzoin oxime (9), the authors feel that further investigation of the completeness of this precipitation and other possible causes of this difference is a separate problem and beyond the scope of this paper.

A modification of the chemical procedure must be employed

in the analysis of columbium-bearing steels, since interference exists between tungsten and columbium under the discharge conditions employed and with the dispersion and resolution available with the authors' spectrograph. However, tungsten and columbium are not ordinarily easily separated (5), so that a modified chemical procedure was developed to permit such separation. The use of phosphoric acid in the modified chemical procedure causes a partial but proportional precipitation of tungsten in its separation from columbium. The working curve drawn up from this method (Figure 2, B), is therefore different from the curve (Figure 2, A) constructed from the original procedure.

This modification is applicable to all steel alloys, eliminating the use of two curves. However, it is advisable to avoid unnecessary loss of tungsten when columbium is not present. This

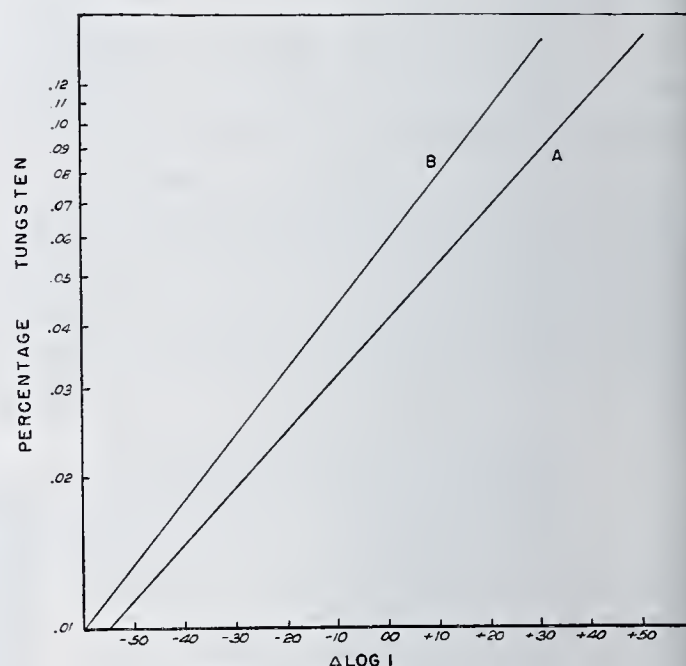


Figure 2. Working Curves

A. Corrosion-resistant steel, carbon tool steel  
B. Columbium-bearing steel

is important when we consider that the amount of tungsten present in the solution to be analyzed is very small—i.e., 0.01% tungsten based on a 2-gram sample is equivalent to 0.0002 gram of tungsten. Furthermore, the number of columbium-bearing steels analyzed for small amounts of tungsten is negligible in proportion to the total number of samples received in this laboratory.

### ACKNOWLEDGMENT

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THIS paper is not to be construed as an official method of the Navy Department.

# Amino Acid Analysis of Some Common Vegetables

## Method for Carbohydrate-Free Extraction of Nitrogen from Fresh Vegetables

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Fresh vegetables (0.5 to 1.0 kg.) were frozen, cut to suitable size, and immersed in acetone at room temperature for 48 hours, then submitted to a continuous acetone extraction for 24 hours. The two acetone fractions were combined and the nitrogenous products washed out by this treatment were set aside for analysis after the removal of acetone, lipids, and plant pigments. The vegetable residues were further extracted by 24-hour immersions in each of two 1.5 liters of hot 90% formic acid. These two fractions were

combined and concentrated in vacuo to 1 liter, and the carbohydrates were precipitated by the addition of 2 liters of 95% ethanol and removed by filtration. The filtrates, which contain the bulk of the nitrogen, were distilled in vacuo to remove the alcohol and formic acid. The combined acetone-soluble and formic acid-soluble residues were found suitable for bioassay or on hydrolysis for amino acid analyses, and contained 90 to 95% of the total nitrogen of the fresh products.

THE current state of knowledge regarding the protein composition of vegetable foods has been recently assessed by Pickery (21): "What is needed is a statement of the amino acid composition of the total proteins of these vegetable products. What is to be found in the literature are more or less incomplete and seldom trustworthy tables of the composition of purified samples of the chief protein component." Because of the worldwide depletion of animal proteins and the consequent increased human consumption of fresh vegetable foods brought about by the present crisis, the need for securing this information has become most urgent. Consideration of these circumstances and the newer knowledge on the effect of deficiencies of certain amino acids in man and experimental animals (1, 2, 3, 5) led the authors to initiate a program of study in 1942, with the purpose of obtaining data on the essential amino acid content of whole fresh vegetables.

The ultimate cause of the lack of suitable data on the amino acid composition of vegetables is the lack of a method for the complete and carbohydrate-free extraction of proteins from vegetable products. It would seem that all that is required is to submit samples of the vegetables directly to hydrolysis and to perform amino acid analyses on the hydrolyzates. The shortcomings of this direct approach became obvious when it was pointed out that acid hydrolysis of proteins in the presence of carbohydrates, which are inevitably found in all foodstuffs, results in the loss of a considerable portion of the protein nitrogen in the form of a black insoluble product known as "humin". The origin of humin nitrogen has been the subject of much study. Thus, Portner and Blish (10) demonstrated that all the tryptophan is lost in this form. Tristram (20) and others (19) have reported the destruction of arginine, histidine, and lysine proteins through acid hydrolysis in the presence of carbohydrates. The loss of proline, cystine, and methionine through the same mechanism has been demonstrated by Lugg (14). Kuiken and co-workers (12) have recently reported significant losses of valine, leucine, and

isoleucine in casein when hydrolyzed in the presence of carbohydrates. In view of this evidence, it would appear impossible to secure accurate information on the amino acid composition of the vegetable by the direct hydrolysis technique.

The alternative solution of the problem lies in the isolation and purification of the protein moiety of the vegetable. Previous attempts at this approach have been notably unsuccessful.

The neutral saline extraction technique of Osborne (17), which proved so useful in his study of the seed proteins, was found ineffective when applied to fresh vegetables. The success of the maceration-extraction technique, also tried by Osborne and Wakeman (18), was hampered by filtration difficulties. In 1923, Chibnall (7) achieved the extraction of 40% of the total nitrogen of some leaf proteins by the aqueous ether treatment. However, he later found these preparations contaminated with pentosans and withdrew his analyses (8). Methods using combinations of various solvents (11) and enzymic removal of the carbohydrate (9) have been applied with some success to seed meals but are not readily adaptable to the study of fresh products. Mazur and Clarke (15) have shown in their study of the marine algae that a carbohydrate-contaminated preparation containing 60 to 90% of the total nitrogen could be conveniently obtained by extraction with 90% formic acid.

After numerous experiments with modifications of the various schemes suggested by these earlier attempts, the authors found that the carbohydrate contaminants of the formic acid extracts of the vegetables prepared as described by Mazur and Clarke could be quantitatively removed by the addition of ethanol without loss of nitrogen. The final product resulting from the isolation procedure evolved on the basis of this finding was found suitable for rat-feeding experiments or on acid hydrolysis for amino acid analysis and contained 90 to 95% of the total nitrogen of the fresh vegetable.

### EXPERIMENTAL

SOLUBILITY OF PROTEINS AND CARBOHYDRATES IN FORMIC ACID AND FORMIC ACID-ETHANOL MIXTURE. Preliminary ex-



Table I. Solubility of Biological Products in 90 Per Cent Formic Acid

Class	Substance	Type of Compound	Solubility at 20-25° C.	
			90% formic acid	90% formic acid, 1 volume + 95% ethanol, 2 volumes
			Grams per 100 Cc.	
Carbohydrates	Cellulose	$\beta$ -Glucosan	0.00	0.00
	Cornstarch	$\alpha$ -Glucosan	0.00	0.00
	Lintner starch	$\alpha$ -Glucosan (modified)	0.00	0.00
	Dextrins	Derived $\alpha$ -glucosan	0.13	0.00
	Sucrose	Disaccharide	5.60	4.50
	Dextrose	Monosaccharide	6.65	5.10
	Gum arabic	Pentosans + hexosans	0.00	0.00
	Gum tragacanth	Pentosans + hexosans	0.00	0.00
	Agar-agar	Galactans + mannans + fucosans	1.98	0.00
Proteins	Zein	Prolamin	7.25	7.25
	Gelatin	Albuminoid	5.90	5.90
	Hemoglobin	Chromo-histone	3.64	3.64
	Lactalbumin	Albumin	0.51	0.51
	Casein	Phosphoprotein	3.07	3.07

periments demonstrated that the formic acid extraction of the fresh vegetables after acetone fixation yielded high nitrogen recovery with an unavoidable impurity of a polysaccharide nature. The observation that the addition of 2 volumes of ethanol to 1 volume of formic acid extract of spinach leaves resulted in the quantitative precipitation of these carbohydrates with only a negligible loss of nitrogen prompted investigation of the solubility of some carbohydrates and proteins in formic acid and formic acid-ethanol mixture.

To 0.2- to 1.0-gram samples of various carbohydrates dried to constant weight in 15-cc. centrifuge tubes are added 10 cc. of 90% formic acid. After thorough mixing the tubes are stored in a 60° oven for 24 hours, being removed and shaken mechanically for 20 minutes at 10 intermittent intervals during the period. At the end of this time, the volume is adjusted to the original level and the tubes are centrifuged. The supernatant solution is decanted and saved and the residues are dried to constant weight in the vacuum desiccator over calcium chloride. The solubility of the carbohydrates per 100 cc. of formic acid is estimated by multiplying by 10 the weight loss incurred by the original sample (Table I). These values were checked by the weight of residues obtained by desiccation of the respective supernatant solutions. Of the carbohydrates tested only dextrose, sucrose, dextrins, and agar-agar are soluble in formic acid. The addition of 2 volumes of 95% ethanol to 1 volume of formic acid solutions of these four carbohydrates results in the quantitative precipitation of dextrins as agar-agar, but not of dextrose or sucrose.

The solubility of the proteins in formic acid is more conveniently estimated from the nitrogen content ( $N \times 6.25$ ) of the supernatant solutions obtained by application of the previously described procedure to protein samples. It is clear from the data so obtained (Table I) that these proteins are equally soluble in formic acid and the formic acid-ethanol mixture. Since the polysaccharides appear to be the principal carbohydrate contaminants of the fractions resulting from the formic acid extraction of the vegetables, the solubility of the proteins and insolubility of the polysaccharides in the formic acid-ethanol mixture affords a surprisingly simple method for the purification of the protein moiety.

**PROCEDURE FOR EXTRACTION OF PROTEINS FROM FRESH VEGETABLES.** From 0.5 to 1 kg. of the fresh vegetable is frozen by storage in the freezing compartment of an electric refrigerator set at 0° C. This serves to break down the cell walls, thereby increasing cell permeability to solvents. The vegetable is then sectioned in preparation for sampling and extraction. (Leafy vegetables are sliced in 1-cm. cross sections; cabbages and beans are diced; potatoes, carrots, and turnips are shredded.) Six 100- to 200-mg. aliquots of this product are weighed out immediately on the torsion balance and total nitrogen is determined by micro-Kjeldahl method (16). Another 100-gram aliquot is set aside for the preparation of the alkaline hydrolyzate needed for estimation of tyrosine and tryptophane as described by Lugg (13).

The remainder of the prepared vegetable is placed in a 2-liter (0.5-gallon) food jar and covered with acetone, U.S.P., and after 2 days the acetone is removed by decantation and saved. The vegetable is then transferred to a fine-mesh cloth sack and submitted to a continuous acetone extraction for 24 hours in the modified Soxhlet apparatus shown in Figure 1, using the 600-watt hot plate, A. At the end of this time the acetone is removed from the extractor and combined with the first acetone extract. The acetone of the combined fractions is recovered by distillation, the insoluble coloring matter and lipids are filtered out, and nitrogen content of the aqueous residue is determined by micro-Kjeldahl method. This nitrogen which is extracted by the acetone treatment results from the mobilization of plant juice incident to dehydration and for want of a better term is called the acetone-soluble fraction. The low-protein nitrogen content of these fractions is indicated by the nitrogen content of precipitate obtained on the addition of 25 cc. of 10% trichloroacetic acid to 15 cc. of sample (Table II). Moreover, since 75 to 85% of the total nitrogen of these fractions occurs as  $\alpha$ -amino nitrogen (4), the amino acids of these fractions are determined directly without hydrolysis and by special techniques to circumvent the interference of the carbohydrates present (to be published).

Since excessive decomposition of formic acid at its boiling point prevented the use of a continuous extraction technique, the acetone-extracted vegetable is treated as follows: The sack and contents are removed from the extraction chamber, C, and exposed to an air current for several hours to drive off the acetone. The incased vegetable is returned to the chamber, which is now filled with 90% formic acid to just below the discharging level. The temperature of the chamber is maintained at 75° to 80° C. by means of a 100-watt lamp, B, and the extraction is continued for 24 hours. This first portion of formic acid is discharged into the boiling flask by the addition of formic acid to a level above the siphon tube and the extraction continued for another 24 hours with a new portion of formic acid. This second formic acid extract is also discharged into the boiling flask. Two such operations suffice to extract almost all the available nitrogen.

The fibrous residue from both acetone and formic acid extractions is transferred to a 2-gallon enameled pail and mixed mechanically for 30 minutes with 2 liters of 95% ethanol to facilitate handling and subsequent drying of the product. The solid matter is removed by gravity and suction filtration, air-dried, and analyzed for nitrogen. The alcohol filtrate is made to 2 liters and added to the combined formic acid fractions which have been previously concentrated in vacuo to 1 liter. The mixture is then allowed to stand for 2 hours at room temperature and the precipitated carbohydrates are filtered out first by gravity through fluted paper and finally by suction in a Büchner funnel. The alcohol and formic acid of these filtrates are removed by

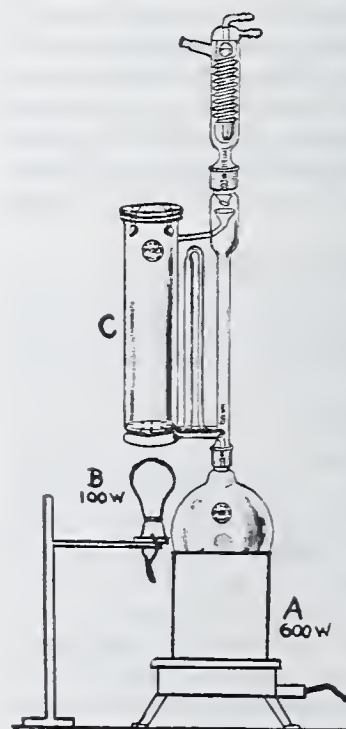


Figure 1. Extraction Apparatus  
A, 600-watt hot plate; B, 100-watt electric lamp; C, extraction chamber

distillation in vacuo. These residues were found to be uniformly carbohydrate-free by the Benedict, Molisch, and iodine tests and constitute the formic acid-soluble fraction of the vegetable nitrogen. They are suitable at this point for rat feeding experiments. Or, they are made to 125-cc. volume with water and, after the addition of 75 cc. of concentrated hydrochloric acid, hydrolyzed by boiling under reflux in an all-glass apparatus for 24 hours. The excess acid is removed by concentrating the hydrolyzate in vacuo to a thick sirup three times successively after the addition of water. The final product is made to 250 to 300 cc. (pH 1 to 2), total nitrogen is determined, and then the humin is filtered out. The humin is extracted with 150 cc. of boiling water and the extract combined with the original filtrate. This final solution is concentrated in vacuo to 200 cc. and then submitted to nitrogen and amino acid analysis. The humin is air-dried and analyzed for nitrogen. A flow sheet of the procedure is given in Chart I.

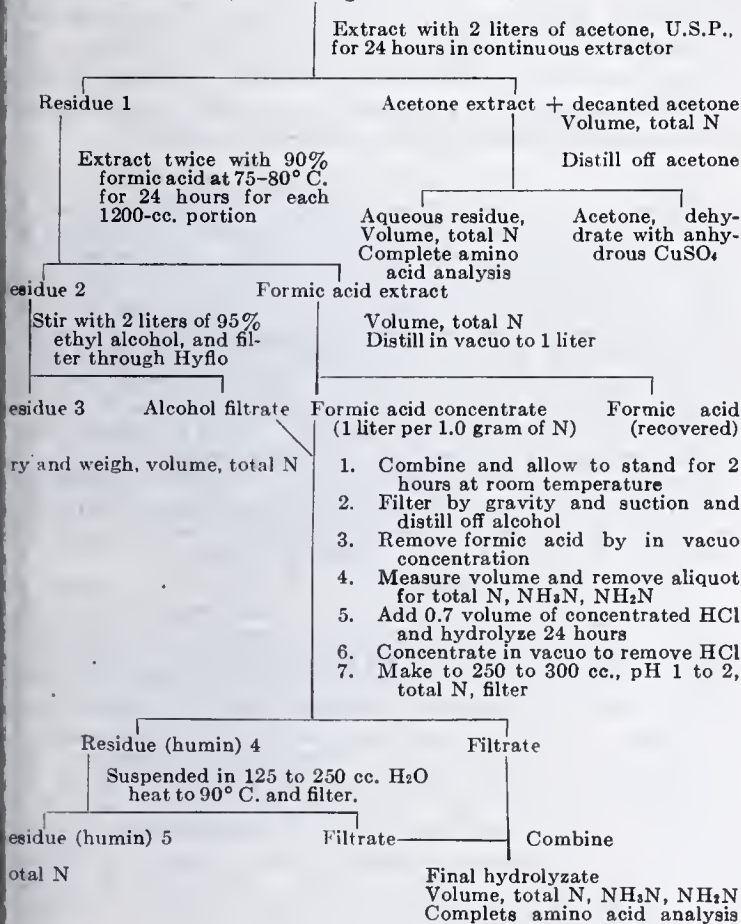


Table II. Distribution of Nitrogen in Fresh Vegetables

Vegetable	String Beans	Carrot	White Potato	White Turnip	White Cabbage	Chinese Cabbage	Kale	Spinach	Celery (Pascal)
Origin	Md.	Calif.	Idaho	N. J.	Md.	Ill.	Md.	N. Y.	Calif.
Sample analyzed, grams	828	1050	1044	890	791	888	590	1290	127½
Total N, grams	2.35	1.33	3.72	1.55	1.36	1.75	3.12	5.43	1.78
Protein (N × 6.25), % Found	1.78	0.79	2.24	1.08	1.07	1.23	3.29	2.63	0.87
U. S. Dept. Agr. (6)	2.4	1.1	2.0	1.1	1.4	1.4	3.2	2.3	1.0
Nitrogen extracted									
Grams	2.1	1.24	3.54	1.52	1.34	1.68	2.86	5.34	1.63
%	90.7	92.5	95.1	97.9	98.6	96.2	91.4	98.2	92.0
Acetone-soluble N, grams	0.57	0.24	1.32	0.33	0.37	0.63	0.48	1.10	1.09
Acetone-soluble N, % of total N	24.2	18.0	35.5	21.2	27.1	36.2	15.4	20.2	61.4
Protein-N in acetone-soluble fraction, mg.	13.2	14.7	22.0	13.6	31.0	19.3	5.3	24.1	8.4
Formic acid-soluble N, grams	1.54	0.99	2.22	1.19	0.97	1.05	2.36	4.24	0.54
Formic acid-soluble N, % of total N	66.5	74.5	59.6	76.7	71.5	60.0	76.0	78.0	30.6
Residue N, gram	0.249	0.095	0.183	0.025	0.018	0.063	0.261	0.093	0.154

Chart I. Flow Sheet of Nitrogen Extraction Procedure for Amino Acid Analysis of Fresh Vegetables

Freeze vegetable, section, weigh (1 kg.), determine total N, and store in acetone 48 hours  
Decant and save acetone, transfer vegetable to cloth sack



APPLICATION OF THE METHOD TO SOME VEGETABLES. The vegetables needed for this study were obtained from retail stores and only the edible portions used. The data on nitrogen distribution are recorded in Table II. Inasmuch as the protein content of the vegetables is a function of numerous variables, the protein content (calculated as N × 6.25) of the specimens is tabulated for comparison with that reported for the vegetable by the U. S. Department of Agriculture (6).

COMMENTS

The authors' data on the solubility of various carbohydrates in formic acid point to the dextrins and mucilages as constituting the

principal impurities in the formic acid extracts of the vegetables. In 1939 circumstantial evidence led Lugg (14) to point out these substances as the possible contaminants in his leaf protein preparations. He was unable to remove them by isoelectric precipitation. It is clear from the present study that the solubility differences in the formic acid-ethanol mixture afford an effective means of separating these carbohydrates from the proteins. However, it is wholly probable that in the study of other vegetable foodstuffs the carbohydrate-protein relationships may be such as not to be separable by this technique.

The authors' experiences with the application of the formic acid-ethanol method to dried seed meals or pulverized dehydrated vegetables were unsatisfactory owing to the formation of gels and loss of dispersion of protein achieved in these preparations. Attempts to overcome these difficulties by the use of fillers such as Celite or filter paper were not completely successful. In the best experiments of this series only 60 to 70% of the total nitrogen could be removed after 5 extractions with formic acid. It appears, therefore, that the adaptability of the method to other foodstuffs is limited by the particle size and physical characteristics of the product.

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# Determination of Soluble Silica in Very Low Concentrations

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**M**ODERN high-pressure central stations are equipped to supply steam of very high quality. Loss of turbine efficiency and capacity because of turbine blade deposits is therefore not expected in such installations. Nevertheless, many high-pressure plants have experienced blading deposits, which have often contained a high percentage of silica. The appearance of such deposits on the blades of a 1250-pound superposed turbine, as well as on the blades of the associated 300-pound units, at one station initiated tests to relate boiler water constituents to steam silica content. Obviously such a study necessitated very reliable determinations of soluble silica in dilute concentrations. The development of an acceptable silica determination procedure by some modification of published methods is shown in this paper.

## SILICA BY SULFITE REDUCTION OF SILICOMOLYBDATE

The plant laboratory had for some time been using rapid colorimetric methods whenever possible for feedwater and boiler water control testing, a Coleman Universal spectrophotometer being utilized for the purpose. Silica in the boiler water was determined by the Kahler (2) method, measuring concentration of silica by means of the blue reduction color of silicomolybdate. With this procedure sulfite is used for reduction, and pH control is used to prevent phosphate interference. Concentration-transmittance curves for the method were developed at a wave length of 700 millimicrons with both 18-mm. and 40-mm. cell optical depths. The curves are linear, 70% transmittance being equivalent to 7.5 and 2.4 p.p.m., respectively.

To adapt the test method at hand to steam analysis, it was only necessary to broaden out the p.p.m. scale of the 40-mm. cell depth curve, making it readable to 0.1 p.p.m. of silica. This sensitivity appeared to be ample for the expected silica values in the steam. Distilled water for reagents was purchased outside the plant. It was assumed that very low concentrations of orthophosphate, with low concentrations of silica, would cause no interference. Hard-rubber bottles were used for all reagents. Pyrex 250-ml. glass-stoppered bottles were used for sample collection.

Subsequent use of the method brought out some practical difficulties. At this very low range of silica concentration the effect of color progression was found to be critical. The rapid color development required measuring the 1-minute sulfite reduction time with stop-watch accuracy. It became evident that the stable life of the reagents was short, the molybdate reagent being dependable for only 8 hours when used for determining small silica values. New bottles, or bottles freshly cleaned with dichromate cleaning solution, were found to cause erroneous silica determinations. Accordingly, separate bottles were provided to sample each point being tested and were used only for that purpose.

**REAGENTS, SULFITE METHOD (2).** Hydrochloric acid, 0.248 *N*; ammonium molybdate solution, 102 grams per liter; and sodium sulfite, 170 grams per liter.

**PROCEDURE.** Treat a 10-ml. sample with 5 ml. of acid and 5 ml. of ammonium molybdate solution. Reduce within 1 to 5 minutes with 10 ml. of sodium sulfite solution. Read % *T* in spectrophotometer 1 minute after reduction at 700  $\mu$  with 18-mm. or 40-mm. cell. Use sample reference solution containing the acid and sulfite plus 5 ml. of distilled water. Determine p.p.m. of silica from the proper *C-T* curve.

**A method for the determination of soluble silica in very pure central station steam or condensate is discussed applicable to the determination of silica in low concentrations (or in small samples) in any water. Its sensitivity allows Nessler tube comparison for values of silica as low as 0.02 p.p.m. A procedure for developing temporary molybdenum blue color standards of long stability is presented.**

## SILICA BY AMINO ACID REDUCTION OF SILICOMOLYBDATE

As the carry-over study progressed values of less than 0.1 p.p.m. of silica became important. It was learned that an increase of the sample volume to 50 ml. made the determination more sensitive and that the method used by Lindsay and Bielenberg (4) resulted in still greater sensitivity. This latter method prevents phosphate interference by destroying the phosphomolybdate complex with sodium citrate. A mixed sulfuric acid and ammonium molybdate reagent proved to be stable. The blue reduction color was obtained by using 1-amino-2-naphthol-4-sulfonic acid reagent rather than sodium sulfite alone. The reducing agent proved stable when made up from recently purchased chemical. The strength of the sodium citrate reagent did not change. Color progression of the reduced silicomolybdate was slight after 1 minute. The test was sensitive to 0.01 p.p.m. of silica. Because of these several advantages the procedure was adopted at this time and used for completion of the silica carry-over study.

Continued use of the method indicated that distilled water purchased in 5-gallon bottles could not be depended upon for constant silica content. Probably the method of cleaning left these bottles unstable as far as silica pickup was concerned. A source of water nearly silica-free and constant was found in the plant. Water obtained by condensing the vapor from the vent of an evaporator condenser, collected and stored in a common 5-gallon bottle and used only for the one purpose, remained nearly stable at about 0.03 p.p.m. of silica. The *C-T* curves developed at 700 millimicrons are not quite linear, for either the 19-mm. cell (ordinary test tube) or the 40-mm. cell. With this method 70% *T* is equivalent to 1.8 and 0.7 p.p.m. of silica, respectively.

**REAGENTS, AMINO ACID METHOD (4).** Sulfuric Acid-Molybdate Reagent. Dissolve 75 grams of c.p. ammonium molybdate in 800 ml. of silica-free water, add 60 ml. of concentrated c.p. sulfuric acid, cool, and make volume up to 1 liter.

**Sodium Citrate Reagent.** Dissolve 430 grams of sodium citrate, U.S.P., in silica-free distilled water and make up to 1 liter.

**1-Amino-2-naphthol-4-sulfonic Acid Reagent.** (A) Dissolve 9 grams of sodium bisulfite in 800 ml. of silica-free distilled water. (B) Dissolve 7 grams of anhydrous sodium sulfite in approximately 100 ml. of silica-free distilled water. To solution B add 1.5 grams of 1-amino-2-naphthol-4-sulfonic acid, mix until dissolved, and add to solution A. Make up total volume to 1 liter.

**PROCEDURE.** Treat a 20-ml. sample with 2 ml. of the amino molybdate reagent. After 5 minutes, add 4 ml. of sodium citrate and mix. Reduce with 1 ml. of the amino acid reagent. Read % *T* in the spectrophotometer after 1 minute at 700  $\mu$  using either 19-mm. or 40-mm. cells. Add 7 ml. of distilled water to 20 ml. of sample for reference solution. Determine p.p.m. of silica from the proper *C-T* curve.

## RECENT MODIFICATIONS

During the carry-over study it became evident that the sensitivity of the test could be increased. Straub (9) had obtained better sensitivity by maintaining a low pH. The sensitivity of the authors' test was increased by omitting the sodium citrate in phosphate-free samples. A check on the method showed sample pH of 1.96 after the addition of sulfuric acid-molybdate reagent, and 5.0 after the addition of sodium citrate. Since the test appeared so desirable in many respects, an attempt to improve the sensitivity seemed in order.

The literature revealed several pertinent facts. Knudsen, Juday, and Meloche (3) show maximum development of the



yellow silicomolybdate at a pH between 1.6 and 2.0 using sulfuric acid. They verify the mole ratio as being 1  $\text{SiO}_2$  to 12  $\text{MoO}_3$ , and establish that a relatively small excess of molybdate is necessary to ensure completion of the silicomolybdate reaction. These authors use a 100-ml. sample and measure values as low as 0.1 p.p.m. of silica by comparing the yellow color developed.

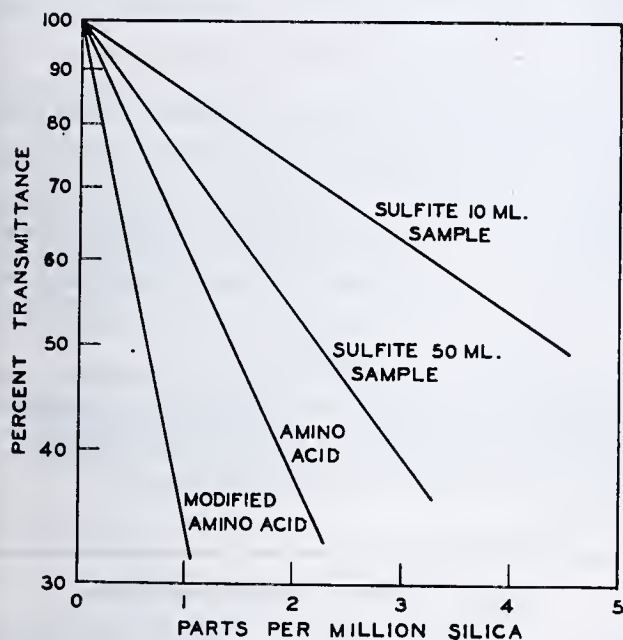


Figure 1. Comparison of Silica Test Methods

The silicomolybdate reaction is indicated as being complete within 5 minutes. Schwartz (6) measures the yellow silicomolybdate color and uses oxalic acid to prevent phosphate interference. He indicates the reaction in destroying phosphomolybdate to be  $\text{MoO}_3$  to 1  $(\text{COOH})_2$ . Straub (9) also has used oxalic acid in reduction method. A statement in the bibliography by Schwartz (7) indicates that tartaric acid or citric acid will prevent phosphate interference. In their work on the molybdenum blue reaction, Woods and Mellon (11) show that in some cases the sulfuric acid-molybdate reagent is superior to hydrochloric acid-molybdate reagent and that chlorostannous acid is a superior reducing agent. Snell (8) suggests the use of excess phosphoric acid to prevent iron and phosphate interference. Thayer (10) indicates that iron must be removed.

From all this information it appeared that a very practical and satisfactory test for low values of silica might be evolved. The phosphoric acid method was eliminated as impractical for rapid routine work. A 100-ml. sample was chosen since the effect of reagents would be less in the larger volume. The mixed sulfuric acid-molybdate reagent was retained because of its known stability and its acceptance by some investigators. A pH check and calculations of the silica-molybdate ratio indicated the advantage of increasing the acidity of this reagent to allow use of 1 ml. per 100 ml. of sample. Because of its expected value in preventing iron interference, tartaric acid was chosen as the reagent to destroy phosphomolybdate, the strength and amount of this reagent to supply an excess being calculated from the relationship  $\text{MoO}_3$  to 1  $(\text{COOH})_2$ . Four milliliters of a 10% solution were found to supply sufficient excess and also to maintain proper pH. One milliliter of the amino acid reagent supplied ample reductant. Concentration-transmittance curves were developed for this method. For both the 18-mm. and 40-mm. optical depths they are linear for the low values being investigated. With this method, 70%  $T$  is equivalent to 0.85 and 0.33 p.p.m. of silica, respectively.

The sensitivity of the chlorostannous acid reductant was checked upon by developing a second 40-mm. optical depth  $C-T$  curve, the amino acid reductant being replaced by the chlorostannous acid as used by Woods and Mellon (11), which was

found to be slightly more sensitive. However, the amino acid was chosen over the chlorostannous acid because of its greater stability.

A silica-free water is required for accurate silica  $C-T$  standards and for making up reagents. Distillation of turbine condensate in a small laboratory still with subsequent storage in a tin-lined tank now supplies a high-grade product. A blank reading for reagents, kept in Pyrex bottles, has not exceeded 0.025 p.p.m. of silica for this modified method. As yet no limit has been found for length of reagent life, reagents as old as 4 months have not exceeded the above blank value. The marked increase in sensitivity of the modified method over previous methods is shown in Figure 1.

Table I. Standard Solution Comparison

Origin of Standard	Comparison	P.p.m. Theoretical	% $T$	P.p.m. from Gravi-metric Curve	% Deviation from Gravi-metric Curve
A.P.H.A. colorimetric, 9-23-41	10 ml. of sulfite	1.0	87.0	0.9	-10
Same, 9-30-42	10 ml. of sulfite	1.0	87.0	0.9	-10
Gravimetric <sup>a</sup>	10 ml. of sulfite	1.0	86.0	1.0	...
Gravimetric	Amino acid	1.0	61.0	1.0	...
Purchased <sup>b</sup> 6-8-43	Amino acid	1.0	61.0	1.0	0
Same, 1-2-44	Amino acid	1.0	61.0	1.0	0
Same, 1-2-44	Modified	0.40	65.2	0.40	...
$\text{Na}_2\text{SiO}_3 \cdot 9\text{H}_2\text{O}$ C.P., fresh, 1.255 grams + 1 gram of $\text{NaOH}/250$ ml.	Modified	0.40	63.2	0.43	+ 7.50
Same, old	Modified	0.40	63.5	0.425	+ 6.25
$\text{Na}_2\text{SiO}_3$ , commercial <sup>c</sup> , 0.5083 gram + 1 gram of $\text{NaOH}/250$ ml.	Modified	0.40	64.5	0.41	+ 2.50

<sup>a</sup> In hard-rubber container.

<sup>b</sup> W. H. & L. D. Betz, rosin-lined container.

<sup>c</sup> Cowles Detergent Co. (Drymet), barrel previously opened.

#### EFFECTS OF IONS AND CONDITIONS ON THE MODIFIED METHOD

Orthophosphate may be present in steam as a result of some boiler operating disturbance. It was found that tartaric acid entirely prevents interference of this ion in any concentration up to the theoretical limits of the reagents. Other organic acids, such as citric or oxalic, are known to be equally effective.

The rate of color development after reduction is shown in Figure 2. It is apparent that the time required for complete color development increases with concentration. Twenty minutes is considered ample time within the proper temperature range. A  $C-T$  curve made up without regard to complete color development will tend to deviate from the linear.

Silica standard solutions were investigated to determine their accuracy and stability. Solutions used in developing the preceding curves, one made as described in the literature (11) and another made from a commercial anhydrous sodium metasilicate, are compared in Table I. The solution standardized by the A.P.H.A. method (1) using the chromate standards was found somewhat low. The nonhydrate ( $\text{Na}_2\text{SiO}_3 \cdot 9\text{H}_2\text{O}$ ), whether fresh or old solid, was found to deviate considerably from the theoretical value. The other solutions were acceptable as standards. Solutions have been found stable in a hard-rubber container for at least one year and in rosin-lined containers for at least 7 months. Investigators have shown (5) that silica solutions thus stored will not deteriorate.

Variations in sample temperature were found permissible in a range at least  $10^\circ$  plus or minus a normal  $75^\circ$  F. laboratory temperature. The temperature effect is shown in Table II.

Iron, present in high quality steam and condensate, is normally in concentrations less than 0.1 p.p.m. Neither ferrous nor ferric iron alone causes interference in this modified procedure. The effect of iron alone, iron with phosphate, iron with silica,



Table II. Effect of Sample Temperature

Sample Temperature ° F.	Theoretical P.p.m.	Curve P.p.m.	Difference P.p.m.
62	0.200	0.192	-0.008
69	0.200	0.192	-0.008
84	0.200	0.204	+0.004
92	0.200	0.213	+0.013
87	0.100	0.084	-0.016
73	0.100	0.100	0
93	0.100	0.085	-0.015
93	0.050	0.048	-0.002
68	0.050	0.040	-0.010

pounds makes it important that the blank reference consisting of 100 ml. of sample and 6 ml. of distilled water be used. As may be seen in Table IV, no practical interference from the organic compounds investigated is then evident.

MODIFIED METHOD REAGENTS. Sulfuric Acid Molybdate Reagent. To 75 grams of ammonium molybdate dissolved in silica-free water, add 322 ml. of 10 N sulfuric acid and make up to 1 liter.

Tartaric Acid Reagent, 10 grams of tartaric acid added to 100 ml. of silica-free water.

1-Amino-2-naphthol-4-sulfonic Acid Reagent. Same as for the previous amino acid reduction method.

Phosphate Solution for Removing Molybdate, 5.02 grams of potassium dihydrogen phosphate per liter.

PROCEDURE. To 100 ml. of sample add 1 ml. of acid-molybdate reagent. After 5 minutes add 4 ml. of tartaric acid solution and mix. Reduce with 1 ml. of the amino acid solution. Read % T in spectrophotometer after 20 minutes (10 minutes for values less than 0.1 p.p.m.), at 700 mμ with 18-mm. or 40-mm. cell. For reference solution use sample plus 6 ml. of water. Determine p.p.m. of silica from proper C-T curve.

A periodic silica determination on 100 ml. of silica-free distilled water supplies a blank value showing the effect of reagents.

Note. When it is desired to remove excess molybdate with phosphate solution, add 1 ml. of the phosphate reagent at least 1 minute before treatment with the tartaric acid.

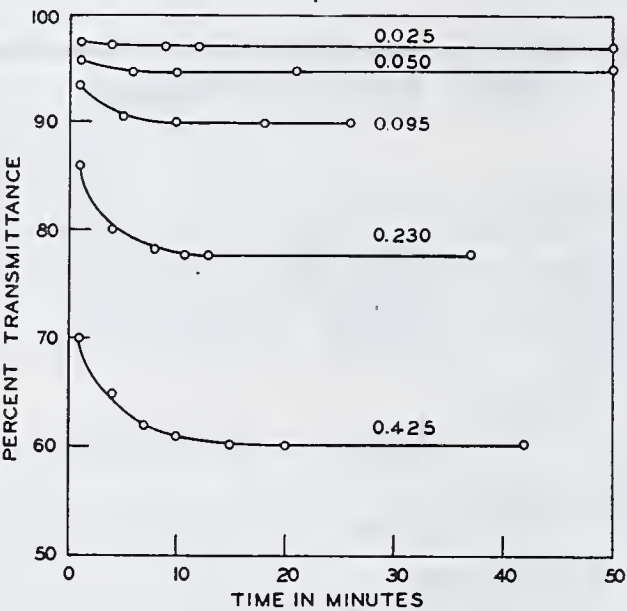


Figure 2. Color Development at Varying P.P.M. Concentration

and iron with both phosphate and silica is shown in Table III. The combination of ferrous iron and phosphate cannot be tolerated since the iron reduces any phosphomolybdate that is formed. The presence of this combination in steam or condensate is unlikely.

The effect of organic compounds was investigated because of academic interest. The highly colored nature of these com-

Table IV. Effect of Organic Compounds

Silica			Organic Compound Present	
Present P.p.m.	Found P.p.m.	Difference P.p.m.	Lignin derivative P.p.m.	Chestnut tannin P.p.m.
0	0.01	0.01	10.0	..
0	0	0	..	5.0
0	0.01	0.01	..	10.0
0.05	0.05	0	0.1	..
0.05	0.055	0.005	..	0.1
0.20	0.20	0	0.1	..
0.20	0.20	0	5.0	..
0.20	0.205	0.005	10.0	..
0.20	0.20	0	..	0.1
0.20	0.20	0	..	5.0

NESSLER TUBE COMPARISON

The sensitivity of the modified test allows color matches as low as 0.02 p.p.m. in 50-ml. Nessler tubes. An attempt to prolong the life of temporary Nessler tube standards has proved effective. The method consists of removing, after the silica reaction is complete, all the active molybdate in the treated sample as phosphomolybdate and then destroying the phosphomolybdate with the organic acid being used for that purpose. The resulting molybdenum blue is identical with that obtained when excess molybdate is present. Standards in stoppered 50-ml. Nessler tubes remain stable for long periods. Tubes kept at room temperature, exposed to the sunlight part of the day, are stable at this writing, 18 days after treatment. The tubes are 18 mm. in diameter, permitting comparative readings directly in the spectrophotometer. Visual checks against fresh standard indicate stability to the eye as well as by instrument.

Note: Since the preparation of the original manuscript the molybdenum blue color standards thus developed have been found satisfactory for phosphate color standards as well as for silica standards when kept in sealed comparison bottles.

CONCLUSION

The modified amino acid reduction method for silica determination has proved very satisfactory and highly practical for routine power plant testing of high quality steam. The stability of the color allows samples to be treated in rapid succession and then all measured in a series later. There need be little regard for time after the required 20 minutes for maximum reduction

Table III. Effect of Iron and Orthophosphate

Silica as SiO <sub>2</sub>			Combination Permissible	Ferric Iron Present P.p.m.	Ferrous Iron Present P.p.m.	Phosphate Present P.p.m.
Present P.p.m.	Found P.p.m.	Difference P.p.m.				
0	0	0	Yes	...	0.5	...
0	0	0	Yes	...	1.0	...
0	0	0	Yes	...	2.5	...
0	0	0	Yes	0.5	...	...
0	0	0	Yes	1.0	...	...
0	0	0	Yes	2.5	...	...
0.1	0.1	0	Yes	1.0	...	...
0	0	0	Yes	1.0	...	0.7
0.1	0.1	0	Yes	0.5	...	...
0.1	0.1	0	Yes	...	...	0.7
0.1	0.1	0	Yes	...	...	1.4
0.2	0.2	0	Yes	0.5	...	1.4
0	0	0	Yes	0.5	...	1.4
0	0.1	0.1	No	...	1.0	0.7
0.05	0.09	0.04	No	...	1.0	0.7
0.1	0.1	0	Yes	...	1.0	...
0.2	0.2	0	Yes	...	1.0	...



The sensitivity of the method and long color stability after reduction allow Nessler tube comparison outside the laboratory. The proper preparation and storage of silica-free water is essential when testing for low silica concentration. Test conditions are not critical and no practical interference from ions other than silica has been observed.

#### ACKNOWLEDGMENTS

The author wishes to express appreciation to the Public Service Company of Northern Illinois for permission to present the results of this investigation. He especially desires to thank fellow members of the Production Department for generous assistance in securing test data and in the preparation of the manuscript. The organic compounds supplied by the National Aluminate Company are also acknowledged.

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PRESENTED before the Division of Water, Sewage, and Sanitation Chemistry at the 107th Meeting of the AMERICAN CHEMICAL SOCIETY, Cleveland, Ohio.

# Quantitative Separations with an Exchange Adsorber

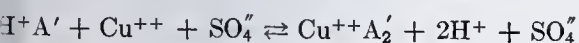
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A SIMPLE quantitative procedure has been devised to separate cations from anions in water solution with a solid ion-exchange adsorber. Separations that demonstrate the possibilities of the method have been made with a precision and accuracy as quantitative as the methods of determination.

The purpose of this work was an examination of the many adsorbers readily available for possible use in analytical chemistry, development of a method for the quantitative separation of inorganic cations from anions in water solution, application of this method to several kinds of cations and anions, and a brief examination of its limitations.

A typical application of the method would be the separation and subsequent determination of copper and sulfate in a solution of copper sulfate. An equation for such a separation would be:



where  $A'$  is one equivalent of adsorber. The  $Cu^{++}A'_2$  quantitatively separates the copper from the sulfate. The adsorber is washed with water and the sulfate determined in a solution containing only one cation, hydrogen ion. The copper is removed from the adsorber with hydrochloric acid and determined in the resulting solution.

#### EXPERIMENTAL

A suitable exchange adsorber for quantitative separations must be a reagent chemical. It should also have the following properties: a large adsorptive capacity per gram of adsorber, a rapid exchange of substances between adsorber and surrounding solution, stability, both chemical and mechanical, rapid and quantitative removal of adsorbed substances with a small volume of wash solution, and rapid quantitative removal of adsorbed substances.

Analytical separations of sodium (5, 7, 8), copper (8), iron (5-8), calcium (5-8), chloride (6, 8), sulfate (4, 5, 7, 8), and phosphate (6, 8), included in this work have been made by a similar method. The general properties and uses of many available adsorbers have been described (2, 9).

Many experiments showed Zeo-Karb to be a satisfactory adsorber. The commercial adsorber obtained from the Permutit Company has many common cations on it, especially sodium ion, and it also contains sulfate ion. These must be removed and the adsorber prepared as a reagent chemical with hydrogen ion as the adsorbed cation. This is done by placing the adsorber in the apparatus described below and applying operation 4 for several hours.

**SEPARATION APPARATUS.** The apparatus shown in Figure 1 was designed and developed from work with a 0.1 *N* solution of ferric chloride and proved satisfactory for other separations. Twenty grams of dry adsorber were wet with water and placed on a mat of glass wool in the primary adsorber, *P*, with the tube, *E*, in the center of the adsorber. Five grams of adsorber were similarly placed in the secondary adsorber, *S*. The solutions were run through *P* and *S* with gravity flow and the apparatus is designed to adjust the rate of flow and time of contact to give the desired quantitative separations. Three apparatus were used.

**PROCEDURE FOR SEPARATIONS.** Each separation began with reagent adsorber with only hydrogen ion adsorbed on it.

1. The apparatus was dismantled and washed with water to remove hydrochloric acid, then 100 ml. of water were allowed to run through *P* and 50 ml. of water through *S*. *P* and *S* were connected and 200 ml. of water were run through *P* and *S* as fast as it ran through *S*. Water was added to *P* just to cover the adsorber and then a little more was added to cause siphoning. A volume of 5 to 7 ml. was added each time.

2. A 25-ml. portion of solution was measured in a pipet and 50 ml. of water were added to it. The solution was added to *P* and allowed to run through *P* and *S* as in operation 1. The volume of the pipet used was 24.91 ml.

3. Adsorbers *P* and *S* were then washed with 200 ml. of water as in operation 1. The washings from 2 and 3 were collected together to determine the anion.

4. Twenty-five milliliters of 6 *N* hydrochloric acid were placed in the 250-ml. flask, *F*, and all parts of the apparatus were connected. The hydro-

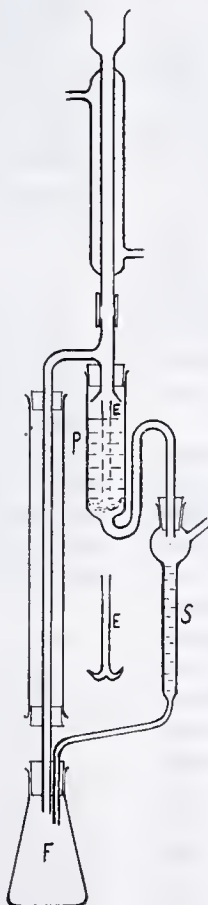


Figure 1



chloric acid was distilled through *P* and *S* for 2 hours. The cation was determined in the solution in flask *F*. Three hours were required to make a separation; for 2 of the 3 hours no personal attention was required.

**REAGENTS.** Analytical reagent chemicals were used and potassium dichromate, sodium oxalate, ammonium dihydrogen phosphate, calcium carbonate, and boric acid were further purified by crystallization.

**SEPARATIONS.** The procedures for the determinations were adapted from textbooks (1, 3). Solutions of ferric chloride, copper sulfate, sodium oxalate, ammonium dihydrogen phosphate, and calcium borate were prepared of determinate or determined concentration. The ions of each solution were separated by the above procedure and the quantity of each ion was determined after the separation. Four or more determinations of each ion were made where necessary in each solution before the separations and the average values were considered the correct values. The precision of the results is the difference between the extreme values over the average value in parts per thousand and the accuracy is the difference between a determined value and the correct value over the correct value in parts per thousand. The results of the separations are given in Table I.

#### DISCUSSION OF RESULTS

The first three results of Table I were secured in an apparatus that had been used many times. The precision of these results for iron, 0.8 part per 1000, compares favorably with the precision of the determinations before the separations, 1.6 parts per 1000. The accuracy is +0.4 to +1.2 parts per 1000. The first three results for chloride have a precision of 0.5 part per 1000 compared with 1.4 parts per 1000 for the original solution. The accuracy of the chloride results is +5.0 parts per 1000. These high results appear to be a property of the apparatus and are not due to incomplete washing. The other results for iron and chloride from the same original solution were obtained from an apparatus that had been used very little. These and many similar results show that a seasoned apparatus is needed to give consistent results of sufficient precision and accuracy.

Two approximately 0.2 *N* solutions of copper sulfate were used. The amount of copper was determined and the same amount of sulfate was assumed present. One solution, 5.117 milliequivalents, gave the first four results in the table and a solution of 5.079 milliequivalents gave the other results. Precisions of the copper results are 1.8 and 1.2 parts per 1000 and the accuracies are -1.8 to 0 and 0 to +1.2 parts per 1000, respectively. The precisions of the results for sulfate are 4.3 parts per 1000 for the first solution and 7.8 parts per 1000 for the second solution. The accuracy of these values is +1.4 to -2.9 parts per 1000 for the first solution and +4.1 to -3.7 parts per 1000 for the second solution. This prevents the use of a constant for the apparatus. The adsorber contains sulfate, so such accuracy is not unexpected.

The determinations of sodium and oxalate after the separations required blanks. The blank for sodium was determined by following the entire procedure and gave an average of 0.0380 milliequivalent of sodium from eight determinations. The wash solution from operation 1 was used for the oxalate blank and gave an average of 0.0220 milliequivalent from nine determinations. The sodium oxalate solution was determinate and the calculated milliequivalent of sodium was 2.492. This shows that the blank for sodium is too small by about 0.014 milliequivalent and that the sodium removed from the adsorber by a blank determination is not the same as the sodium removed when an exchange occurs. The error for sodium is calculated by considering 2.492 milliequivalents the correct value for sodium. A precision of 3.6 parts per 1000 and an accuracy of +3.6 to +7.2 parts per 1000 are obtained. A blank of 0.052 milliequivalent of sodium would give equal precision and accuracy. The errors for the oxalate determinations are calculated from the average value, 2.492

milliequivalents, obtained by comparing the permanganate and oxalate solutions. The precision and accuracy for all the oxalate results are less than 2.0 parts per 1000.

The ammonium dihydrogen phosphate solution was determinate but determined values for ammonium and phosphate were obtained and used as the correct values. The calculated value for milliequivalents of ammonium ion is 2.491 and the determined value is 2.489. The values obtained after the separation show a precision of 1.2 parts per 1000 and an accuracy of +6.0 to +7.2 parts per 1000. This indicates that a constant of 0.01 milliequivalent should be applied and results would be equally precise and accurate. The calculated value for phosphate is 2.492 milliequivalents and the determined value is 2.497 milliequivalents. The precision is 3.6 parts per 1000 and the accuracy is +1.2 to -2.4 parts per 1000.

Table I. Milliequivalents of Ions Found after Separations

(Present in original solution: iron 2.549, chloride 2.018 milliequivalents)									
Fe	Cl	Cu	SO <sub>4</sub>	Na	C <sub>2</sub> O <sub>4</sub>	NH <sub>4</sub>	PO <sub>4</sub>	Ca	BO <sub>2</sub>
2.552	2.027	5.108	5.124	2.506	2.488	2.505	2.500	2.486	2.49
2.550	2.028	5.108	5.107	2.509	2.491	2.504	2.494	2.486	2.48
2.551	2.027	5.117	5.102	2.506	2.490	2.504	2.495	2.494	2.49
2.562	2.018	5.117	5.106	2.501	2.490	2.507	2.496	2.495	2.48
2.558	2.037	5.079	5.097	2.510	2.490	2.504	2.492	2.486	2.49
...	2.032	5.085	5.100	...	...	2.505	2.491	2.494	2.49
...	...	5.079	5.060	...	...	...	...	2.494	2.49
...	...	5.085	5.064	...	...	...	...	...	...

Determinate 0.1 *N* calcium borate, 0.1 *N* calcium chloride, and 0.1 *N* boric acid solutions were prepared and the comparison of the two latter solutions with solutions of potassium permanganate and carbon dioxide-free sodium hydroxide, respectively, gave determined values for the original solutions to compare with values after the separations: 2.492 milliequivalents of calcium and 2.492 milliequivalents of borate. Precision of the calcium results is 3.6 parts per 1000 and the accuracy is +1.2 to -2.4 parts per 1000. Precision of the borate results is 0.8 part per 1000 and the accuracy is -0.4 to -1.2 parts per 1000.

**LIMITATIONS OF THE METHOD.** Conditions for a successful separation are a small total ion concentration and a pH of the solution not less than 2 and preferably about 4. This procedure applied to 0.1 *N* solutions of potassium dichromate, potassium bromate, and potassium iodate gave reduction of the anions. The iodide in a 0.1 *N* potassium iodide solution was oxidized to iodine on the adsorber.

#### SUMMARY

The adsorber Zeo-Karb used in a simple apparatus gave separations of cations and anions in solutions of ferric chloride, copper sulfate, sodium oxalate, ammonium dihydrogen phosphate, and calcium borate as quantitative as the methods used to determine these ions with the exception of sulfate ion. The separations of sulfate are quantitative enough to be useful when other methods are not available. The acid and reducing properties of the adsorber limit the usefulness of this method to ions not adversely affected by these properties.

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# Substitution of Bromine

## When Determining Unsaturation of Straight and Branched-Chain Olefins

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Substitution (evidenced by formation of hydrobromic acid) has been found to occur for a variety of olefins including isobutylene polymers, pentene-2, hexenes, and octene-1, when bromate-bromide reagents are employed for determining unsaturation. Halohydrin formation with generation of an equivalent amount of hydrobromic acid is negligible if the reagents contain a high concentration of bromide ion. Bromine numbers approaching theory have been obtained on some of the compounds studied, such as diisobutylene and trimethylethylene, even though substitution occurs. This is possible only if the departure from the expected reaction is automatically compensated. The bromine substitution product seems to be relatively indifferent to further bromination either by substitution or by addition. While bromine numbers obtained on certain petroleum fractions may be somewhat misleading, the method is still recommended for general analysis until a more direct method is found.

In a previous article (9) the authors presented data showing that the theoretical bromine number of diisobutylene and trimethylethylene could be obtained by employing a modification of the Francis method (2). The modification failed, however, to give theoretical values for the higher polymers, tri- and tetra-isobutylene. Since these data were published, a study has been made to determine whether other procedures would give reliable unsaturation values for all three isobutylene polymers.

The procedures investigated were based on addition of bromine and thiocyanogen iodide to the double bond, and included those of McIlhenny (10, 11), Kaufmann and Grosse-Oetringhaus (?), Ehrig and Levin (13), and certain unpublished modifications of the Lewis and Bradstreet method. All these methods gave inconsistent results, and in all cases except the thiocyanogen iodide procedure extensive formation of hydrobromic acid occurred. The formation of halogen acid is in line with the observations of previous investigators.

Hal'pern (4) states that bromometric analysis by the methods of McIlhenny and of Kaufmann (6, 8) is usually accompanied by evolution of hydrogen bromide, although the main mass of this acid is not necessarily the result of substitution. In a study of pinene with Kaufmann's reagent, Hal'pern concluded that the primary products of interaction of unsaturated compounds with bromine are not 1,2-dibromides. He considers them to be unstable bromides which are rearranged immediately to form the stable dibromides or are decomposed into hydrogen bromide and unsaturated monobromides, with the possibility of the formation of a new compound that would be incapable of adding bromine.

Kaufmann (6, 8) utilized a solution of bromine in methanol saturated with sodium bromide for determining bromine numbers of various organic compounds. In verifying the method by determining the total amount of bromine consumed he found the values obtained were always the same as those calculated and he, therefore, assumed that the double bond was saturated without the formation of substitution products. Contrary to such a supposition, Jordan (5) found when titrating styrene and indene by the same method that one half of the bromine consumed was present in the bromination flask as hydrobromic acid.

Francis (3) reported that saturation of double bonds with bromine water gives principally the bromohydrin derivatives and that the relative amount of dibromide formation is just as small with bromine water in 4 N sulfuric acid, in which the concentration of hypobromous acid is negligible, though the rate of saturation is less than one thousandth as great. Terry and Eichelberger (12) have shown that bromohydrin formation can be prevented in the case of sodium maleate and sodium fumarate by a high concentration of sodium bromide.

Buc (1) has reported that in the case of highly branched olefins in the higher molecular weight ranges, the major reaction with halogens in general consists of substitution. He employed reagents saturated with potassium bromide.

Recent data obtained on a number of straight- and branched-chain olefins (including diisobutylene and pentene-2) show that halohydrin formation with generation of an equivalent amount of hydrobromic acid does not occur to any appreciable extent when reagents saturated with potassium bromide are used for measuring unsaturation. It is believed that the major amount of hydrobromic acid found in the following experimental work is due to substituted halogen in the molecule. For the sake of consistency it is thus preferred to use the term "substitution" to account for the total amount of halogen acid determined in each experiment.

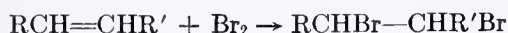
It is obvious that in cases where substitution occurs to any appreciable extent, a measure of unsaturation based on bromine addition will not be reliable. In view of the apparent occurrence of both addition and substitution, a quantitative study has been made of the extent to which each takes place to determine whether conditions exist which will permit substantially complete addition before appreciable substitution occurs.

### DEFINITIONS

In treating the experimental data, it is convenient to consider three bromine numbers (apparent, substitution, and addition) having the following definitions:

1. Apparent bromine number is defined as the total weight of bromine consumed when employing a bromine addition method. The apparent bromine number is equivalent to:

$$\frac{(\text{Total weight of bromine added} - \text{unreacted bromine})}{\text{weight of sample}} \times 100$$



2. Substitution number is defined as the weight of bromine contained in the hydrobromic acid formed during the addition reaction. The substitution number may be expressed as:

$$\frac{\left( \text{Weight of hydrobromic acid} \times \frac{\text{Br}}{\text{HBr}} \right)}{\text{weight of sample}} \times 100$$

3. Addition number is defined as the weight of bromine that is added to a double bond when determining unsaturation by a bromate-bromide method. The addition number is equivalent to:

$$\text{Apparent bromine number} - 2x \text{ substitution number}$$

or

$$\frac{[\text{Total weight of bromine consumed} - 2x (\text{bromine equivalent to hydrobromic acid})]}{\text{weight of sample}} \times 100$$

All three numbers have the dimensions  $\frac{\text{Cg. of bromine}}{\text{Grams of sample}}$

The experimental work consists of a study of addition and substitution of bromine occurring when nascent bromine generated from a bromate-bromide solution is employed. The reactions were carried out under varying conditions described in this paper.



Table I. Addition and Substitution by Bromate-Bromide Method without Excess Acid

Compound	Theoretical Bromine No. Cg./g.	Apparent Bromine No. Cg./g.	Substitution No. Cg./g.	Bromine Consumed during Substitution Reaction Cg./g.	Addition No. Cg./g.	Temperature ° C.
Diisobutylene	142.4	123.1	57.7	115.4	7.70	25
		127.4	60.1	120.2	7.14	25
Triisobutylene	94.9	49.5	19.3	38.6	10.97	25
		43.3	18.9	37.8	5.62	25
Tetraisobutylene	71.2	45.8	19.2	38.4	7.40	25
		56.0	23.6	47.2	8.80	27

#### INVESTIGATION OF SUBSTITUTION WITH BROMATE-BROMIDE REAGENT

It had been suspected for some time that considerable substitution occurred when the unsaturation of highly branched olefins was measured by the authors' method previously published (9). The extent of substitution and addition of bromine has been examined when various modifications of the method have been applied to measurements of unsaturation in isobutylene polymers.

**USE OF EQUIVALENT AMOUNT OF SULFURIC ACID.** The following experiment was conducted to find out if the apparent bromine number could be obtained without the formation of hydrobromic acid by using an exactly equivalent amount of sulfuric acid necessary for completion of the reaction instead of an excess as directed in the original procedure (9). The compounds used were di-, tri-, and tetraisobutylene.

**Procedure.** Twenty milliliters of saturated potassium bromide solution and 15 ml. of *n*-heptane are placed in a 250-ml. glass-stoppered flask, and 1 ml. of sample is added. Sufficient 0.5 *N* potassium bromate for the sample plus 1.2-ml. excess is next added, followed by an exactly equivalent amount of 0.5 *N* acid. The flask is shaken vigorously for 2 minutes, saturated potassium iodide solution added, and the liberated iodine titrated with 0.1 *N* thiosulfate, using the disappearance of the iodine color as an indicator. This titration represents the free bromine. Saturated potassium iodate solution is now added, and any iodine present titrated with thiosulfate. The amount of iodine liberated is equivalent to the hydrobromic acid formed. Apparent addition and substitution bromine numbers are calculated as shown above.

The data given in Table I indicate not only that the apparent bromine number is too low, but also that substitution occurs when excess acid is not present.

**EFFECT OF AMOUNT OF BROMATE SOLUTION ON APPARENT BROMINE NUMBER.** It had been noted in the original method (9) that after addition of a few milliliters of bromate (2 to 4 ml., or a fraction of the volume necessary for theoretical requirements) to samples of isobutylene polymers and subsequent shaking, a faint yellow color was evident. If addition of the reagent was stopped at this point and the sample was shaken for 2 minutes, the yellow color often persisted, indicating an apparent excess. Heretofore the procedure has been to ignore this slight color and continue to add bromate until a strong brownish yellow color is obtained before shaking for the specified 2 minutes. However, in the actual determination of unsaturation in the compounds, for this study, addition of bromate was purposely stopped at the first sign of the yellow color. This was taken as a starting point. The amount of bromate was increased for each successive sample until the volume of bromate represented an excess considerably over the theoretical amount necessary for addition.

**Procedure.** Samples of 0.7 gram were added to 20 ml. of 10% sulfuric acid (saturated with potassium bromide) and 15 ml. of *n*-heptane in a glass-stoppered flask, a specified volume of 0.5 *N* potassium bromate was added, and the mixture was shaken for 2 minutes. Potassium iodide was then added, and the liberated iodine titrated with 0.1 *N* thiosulfate, using starch as an indicator. The bromine number was calculated (cg. per gram), and the results were tabulated.

The values on di-, tri-, and tetraisobutylene, shown in Table II, indicate that some reaction besides addition is taking place otherwise a constant value should be expected after the theoretical amount of bromate has been added.

**EFFECT OF AMOUNT OF BROMATE ON SUBSTITUTION.** The determination of addition and substitution on di-, tri-, and tetraisobutylene was made (1) by considering the sample as an unknown and determining unsaturation by the authors' original method (9), (2) as in (1) except that the theoretical amount of bromate was added, and (3) as in (1) except that the theoretical amount of bromate plus 1-ml. excess was added.

**Procedure and calculations.** Exactly 20 ml. of 10% sulfuric acid (saturated with potassium bromide) must be added to each sample in order to determine the increase of acidity due to formation of hydrobromic acid. A blank on the 10% sulfuric acid is first run by diluting the acid to 100 ml. in a glass-stoppered volumetric flask. A 5-ml. aliquot is pipetted into an Erlenmeyer flask containing 50 ml. of distilled water, and 10 ml. of a saturated potassium iodate solution and 2 ml. of saturated potassium iodide solution are added. The iodine liberated is titrated with 0.1 *N* sodium thiosulfate, and the equivalent acid is calculated as bromine.

Table II. Bromate-Bromide Method Showing Increase in Bromine Number with Increase of 0.5 *N* Bromate Solution

0.5 <i>N</i> KBrO <sub>3</sub> , Ml./g.	(Theoretical Bromine No., Cg./Gram) 142.4 Diisobutylene	94.4 Triisobutylene	71.2 Tetraisobutylene
5	.....	.....	23.0
10	.....	.....	40.0
11	.....	44.0	.....
15	.....	60.0	57.2
20	80.0	75.5	70.3
25	100.0	87.0	80.3
30	120.0	95.0	87.0
35	139.0	100.5	89.8
40	147.7	104.0	90.2
45	149.0	.....	.....
50	150.5	.....	.....

The apparent bromine number as represented by the amount of potassium bromate added is calculated in the usual manner:

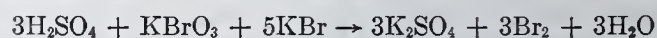
$$\frac{0.08 \times \text{normality of KBrO}_3 \times \text{net ml. of KBrO}_3}{(\text{specific gravity} \times \text{volume of sample})} \times 100$$

The bromine equivalent of the 20 ml. of 10% sulfuric acid saturated with potassium bromide must now be calculated:

$$\frac{\text{H}_2\text{SO}_4}{2} \approx \frac{\text{Br}_2}{2} \approx \frac{\text{I}_2}{2} \approx \frac{\text{Na}_2\text{S}_2\text{O}_3}{1} \approx \frac{\text{KBrO}_3}{6}$$

Therefore  $0.08 \times \text{normality of Na}_2\text{S}_2\text{O}_3 \times \text{ml. of Na}_2\text{S}_2\text{O}_3 \times \text{dilution factor} = \text{total grams of Br}_2 \approx 20 \text{ ml. of 10\% H}_2\text{SO}_4 \text{ (A)}$

In the determination of the apparent bromine number, the addition of bromate requires acid to liberate bromine according to the equation



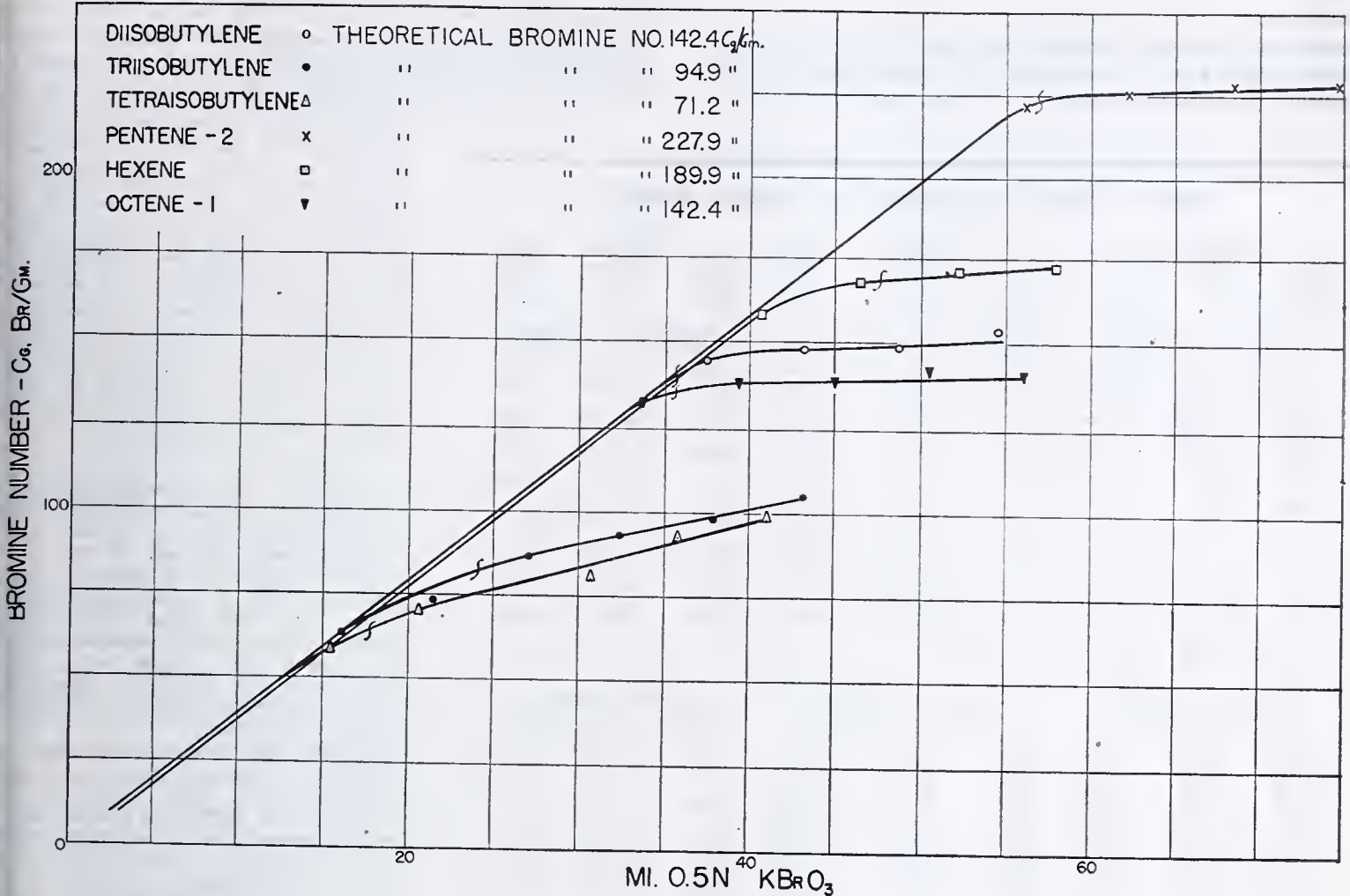
so that the amount of acid used to react with the potassium bromate in terms of bromine will be:

$$0.08 \times \text{normality of KBrO}_3 \times \text{ml. of KBrO}_3 = \text{grams of Br}_2 \text{ equivalent to H}_2\text{SO}_4 \text{ used (B)}$$

Thus the amount of sulfuric acid present at the start of the determination in terms of an equivalent amount of bromine is A - B, or C.

After the specified 2-minute shaking period and titration of excess bromate, the sample is treated in the following manner. The contents of the flask are transferred to a 250-ml. separator funnel and washed thoroughly with water. The water layer is drained off as quickly as possible into a 100-ml. glass-stoppered volumetric flask, the solvent layer is washed several times with distilled water, and these washings are added to the flask. The solution is made up to volume, 5 ml. are transferred to an Erlenmeyer flask containing 50 ml. of distilled water, 10 ml. of a saturated potassium iodate solution are added, and the iodine is titrated with 0.1 *N* sodium thiosulfate. (The addition of potassium iodide at this point is not necessary, since it was added





earlier in the determination.) The total amount of acid present at the end of the determination will be calculated as in the following equation.

$$0.08 \times \text{normality of Na}_2\text{S}_2\text{O}_3 \times \text{ml. of Na}_2\text{S}_2\text{O}_3 \times \text{dilution factor} = \text{grams of Br}_2 \text{ equivalent to H}_2\text{SO}_4 + \text{HBr (D)}$$

The difference, D - C, is the amount of bromine equivalent to the hydrobromic acid formed by substitution.

$$\frac{(D - C)}{\text{weight of sample}} \times 100 = \text{amount of substitution}$$

(in grams of bromine per grams of sample) which took place upon addition of the stated amount of potassium bromate.

Treating the sample as an unknown is necessary in order to find out how much halogen is due to addition as well as substitution when the determination is run in the regular way—i.e., by using 1-ml. excess of potassium bromate. In the present experiments, where the samples were considered as unknown, bromate was added until a brownish yellow color resulted.

The data in Table III show that substitution takes place when the determination is carried out by the bromate-bromide method in the regular manner, and also when this method is modified to the extent of using only the theoretical amount of bromate or the theoretical amount of bromate plus 1-ml. excess.

DETERMINATION OF APPARENT ADDITION AND SUBSTITUTION NUMBERS ON BRANCHED AND STRAIGHT-CHAIN OLEFINS

It has been found that a bromine substitution number can be obtained with or without saturation of the double bond for a wide variety of olefins when using a modification of the bromate-bromide method. This is illustrated by work on di-, tri-, and tetraiso-butylene, pentene-2, hexene, and octene-1.

Changes in the procedure required that all factors except the bromate solution be kept constant in order to follow the course of both addition and substitution. The amount of bromate

Table III. Addition and Substitution Using Bromate-Bromide Method with and without Modifications

	Theoretical Br <sub>2</sub> No. Cg./g.	Ap- parent Br <sub>2</sub> No. Cg./g.	Substitu- tion No. Cg./g.	Bromine Consumed during Substitu- tion Reaction Cg./g.	Addi- tion No. Cg./g.
diisobutylene, regu- lar method (1 ml. excess KBrO <sub>3</sub> )	142.4	144.0 143.6	57.6 56.4	115.2 112.8	28.8 30.8
Theoretical amount of KBrO <sub>3</sub> + 1-ml. excess		144.1 144.1	47.7 53.3	95.4 106.6	48.7 37.5
Theoretical amount of KBrO <sub>3</sub> , no excess		141.8 141.8	55.4 54.3	110.8 108.6	31.0 33.2
triisobutylene, regu- lar method (1ml. excess KBrO <sub>3</sub> )	94.9	73.7 73.7	35.7 34.7	71.4 69.4	2.3 4.3
Theoretical amount of KBrO <sub>3</sub> + 1-ml. excess		86.6 85.9	40.6 41.6	81.2 83.2	5.4 2.7
Theoretical amount of KBrO <sub>3</sub> , no excess		80.4 80.4	38.1 34.9	76.2 69.8	4.2 10.6
tetraiso-butylene, regular method (1 ml. excess KBrO <sub>3</sub> )	71.2	39.9 45.5	19.3 21.4	38.6 42.8	1.3 2.7
Theoretical amount of KBrO <sub>3</sub> + 1-ml. excess		62.9 67.8	28.4 30.4	56.8 60.8	6.1 7.0
Theoretical amount of KBrO <sub>3</sub> , no excess		58.7 63.2	28.0 30.0	56.0 60.0	2.7 3.2



varied from 2 ml. to a volume far in excess of that necessary to obtain the theoretical addition value, and was added in increments of 2 to 4 ml. A value was thus obtained which served to indicate the apparent amount of bromine used for addition at

that particular point, although this may not necessarily be the actual bromine number of the sample. The acid layer was separated and the solvent layer washed with water. The acid solution and washings were made up to 100 ml. and a 5-ml. sample was used for titrating the hydrobromic acid formed by the reaction of bromine on the hydrocarbon. The method of McIlhenny (10, 11)—i.e., reacting the hydrobromic acid with potassium iodate and titrating the resulting iodine with sodium thiosulfate—was followed. The difference between the amount of acid present corrected for its reaction with the bromate solution, and the amount present as found by the thiosulfate titration, is equivalent to the bromine used for substitution.

**Procedure.** Pipet accurately 20 ml. 10% sulfuric acid, saturated with potassium bromide, into a 300-ml. glass-stoppered flask and add 15 ml. of heptane and 1.0 ml. of sample. Add a volume of 0.5 N potassium bromate, stopper the flask, and shake for 5 minutes. Add 5 ml. of saturated potassium iodide and titrate any iodine formed with 0.1 N sodium thiosulfate. This will determine the amount of bromine reacted with the sample. Transfer the contents of the flask to a 250-ml. separatory funnel and wash thoroughly with water. Drain off the water layer as quickly as possible into a 100-ml. glass-stoppered volumetric flask. Wash the heptane (solvent) layer four or five times with small quantities of distilled water, adding the washings to the volumetric flask. Make up to volume and transfer 5 ml. to an Erlenmeyer flask containing 50 ml. of distilled water. Add 10 ml. of a saturated potassium iodate

Table IV. Values for Substitution and Apparent Addition

0.5 N KBrO <sub>3</sub> Ml./g.	Total or Apparent Bromine No. <sup>a</sup> Cg./g.	Substi- tution No. Cg./g.	0.5 N KBrO <sub>3</sub> Ml./g.	Total or Apparent Bromine No. <sup>a</sup> Cg./g.	Substi- tution No. Cg./g.	0.5 N KBrO <sub>3</sub> Ml./g.	Total or Apparent Bromine No. <sup>a</sup> Cg./g.	Substi- tution No. Cg./g.
Diisobutylene, Theoretical Br <sub>2</sub> No. = 142.4 Cg./G.			Triisobutylene, Theoretical Br <sub>2</sub> No. = 94.9 Cg./G.			Tetraisobutylene, Theoretical Br <sub>2</sub> No. = 71.2 Cg./G.		
2.87	11.12	8.11	2.69	10.46	3.30	2.55	9.96	0.013
5.74	22.65	17.07	5.39	21.29	9.82	5.11	20.25	4.09
8.60	34.11	18.77	10.78	42.84	22.83	10.22	40.56	13.31
11.47	45.58	24.66	16.16	63.63	32.65	15.32	57.88	23.55
14.33	57.04	20.51	21.55	73.72	33.95	20.44	70.61	29.70
17.20	68.50	30.86	26.94	87.01	45.89	25.54	77.22	33.81
20.08	79.98	32.29	32.32	93.21	39.99	30.64	81.99	33.33
25.80	102.91	42.95	37.72	98.22	42.93	35.77	92.79	42.29
31.54	125.74	49.12	43.10	104.95	47.09	40.90	98.47	46.13
37.26	146.87	56.44	...	...	...	...	...	...
43.00	148.69	62.64	...	...	...	...	...	...
48.73	149.58	54.89	...	...	...	...	...	...
54.45	154.69	58.30	...	...	...	...	...	...
Pentene-2, Theoretical Br <sub>2</sub> No. = 227.9 Cg./G.			Hexene, Theoretical Br <sub>2</sub> No. = 189.9 Cg./G.			Octene-1, Theoretical Br <sub>2</sub> No. = 142.4 Cg./G.		
3.10	11.92	2.49	2.89	7.89	0	2.80	10.87	12.31
6.21	24.32	4.99	5.78	20.33	0	11.20	44.55	10.34
12.42	49.23	12.47	11.56	43.24	1.49	22.40	89.33	17.36
18.64	74.04	13.74	17.35	67.17	5.11	...	...	...
24.85	98.87	24.94	23.12	90.32	6.42	...	...	...
31.04	123.69	16.28	28.92	113.46	13.48	...	...	...
37.29	148.52	29.96	34.70	136.43	15.95	33.60	132.49	25.47
43.50	173.35	32.38	40.48	159.81	21.86	39.20	139.00	28.69
49.70	198.35	39.97	46.26	168.82	18.29	44.80	140.01	27.46
55.90	222.26	45.08	52.10	172.72	21.05	50.40	143.05	29.02
62.08	225.90	48.96	57.85	173.41	21.50	56.00	141.22	27.65
68.30	228.11	58.98	...	...	...	...	...	...
74.55	228.35	57.64	...	...	...	...	...	...

<sup>a</sup> Apparent addition at any given point.

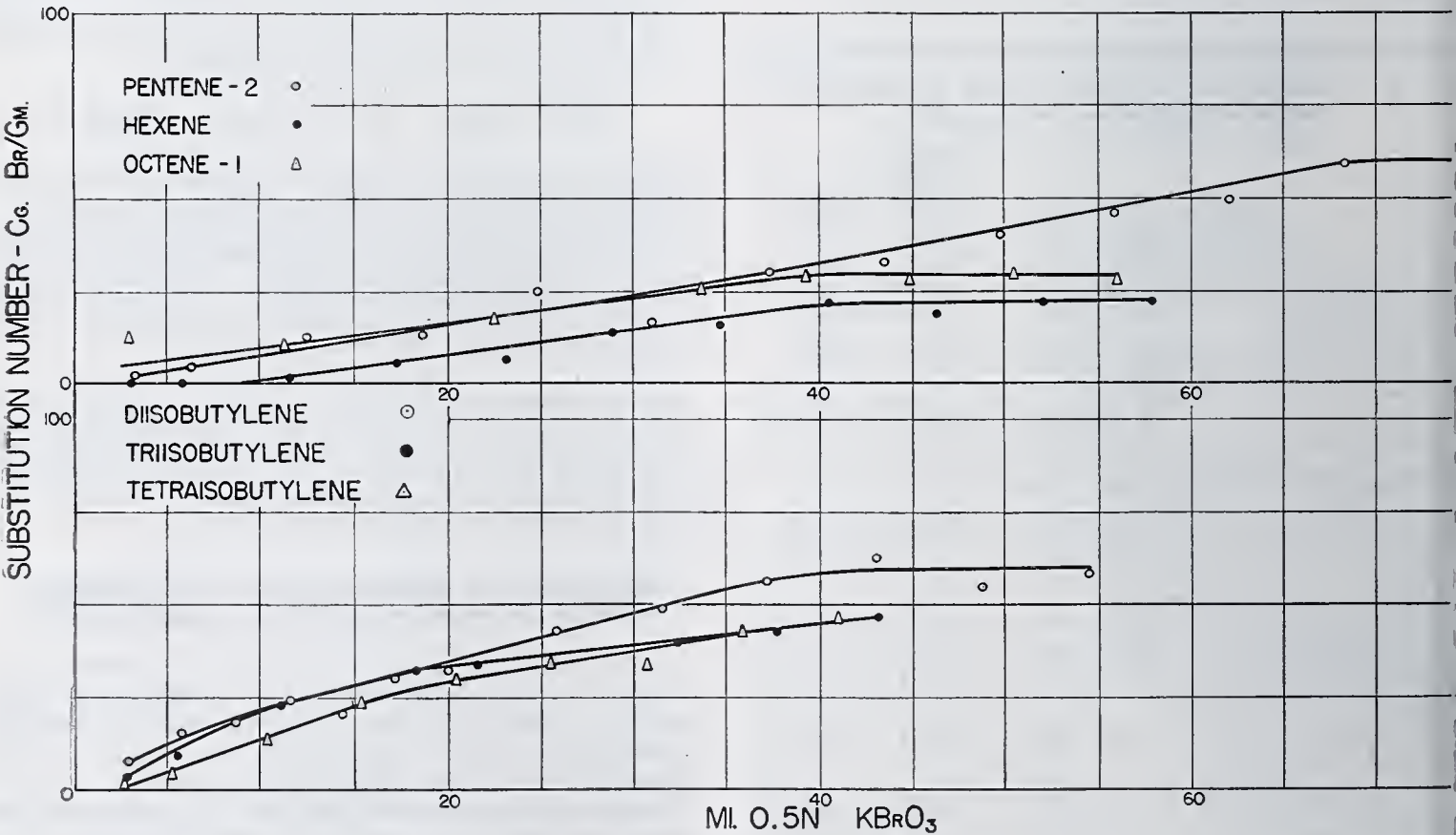


Figure 2. Substitution in Unsaturates



solution, and titrate the iodine with 0.1 *N* sodium thiosulfate. Run a blank on the sulfuric acid.

Repeat the above procedure for every addition of bromate, using 1.0 ml. of sample each time. In this way a series of values for both substitution and addition is obtained which may be plotted against the volume of 0.5 *N* potassium bromate used.

The values for substitution and apparent addition are shown in Table IV. If these values are plotted against the volumes of 0.5 *N* potassium bromate used, curves of the type shown in Figures 1 and 2 are obtained. In most cases the substitution and apparent bromine number curves flatten out at the same point. The apparent bromine number curves begin to level off at the theoretical value, and for all practical purposes reach a constant value regardless of the excess of bromate used. This also applies to the substitution curves.

The data in Table IV show the potassium bromate solution to have been added in uneven amounts. The reagent actually used for the experimental work, although differing somewhat from the required normality, was added in increments of 2, 4, 6, 8, 10, 12, etc., ml. Calculations were made placing these volumes on an exact 0.5 *N* basis.

Calculations show that pentene-2, hexene, octene-1, diisobutylene, triisobutylene, and tetraisobutylene require 57.0, 47.5, 35.6, 23.8, and 17.8 ml., respectively, of 0.5 *N* bromate for supplying the exact amount of bromine needed to saturate the double bond of each compound, based on a 1-gram sample. It is shown in the above data that substitution of bromine takes place after a small fractional volume (2.55 to 3.10 ml.) of the total quantity of the reagent required has been added to any of these unsaturates with the exception of hexene. With hexene, however, substitution value could be obtained after adding 24.3% of the theoretical volume of bromate.

## CONCLUSIONS

Substitution has been found to occur with all the olefins investigated when various modifications of the bromate-bromide method, using potassium bromide-saturated reagents, were employed. While substantially theoretical bromine numbers have been obtained for straight-chain olefins and some branched-chain olefins as shown in the previous paper (9), it is felt that the method is empirical.

In the previously described procedure (9) an excess of 1 ml. of the bromate reagent is used, followed by shaking for exactly 2 minutes. If this method is followed precisely, it will be useful for determining unsaturation of many known compounds having double bonds. However, bromine numbers obtained by the same method on unknown mixtures may be subject to question, in particular if the mixtures contain highly branched olefins. More direct methods, such as hydrogenation, may be more accurate.

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# Absorption Spectra of Volatile Essential Oils Detection of Alpha-Dicarbonyl Compounds

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A test devised for detection of  $\alpha$ -dicarbonyl compounds in fatty materials has been extended to volatile essential oils. Tests on juniper berry; orange peel, and coriander seed oils are reported, as evidence that the flavor value of botanicals may be rapidly and accurately estimated by determination of the extinction values or  $\alpha$ -dicarbonyl content of volatile essential oils used in the manufacture of alcoholic beverages.

THE quality of alcoholic beverages such as gin, cordials, and liqueurs can be maintained only by rigid chemical control and taste tests. Flavor control starts with the correct selection of botanicals and other materials used for their flavor value. Specifications used for the purchase of botanicals are chemical constants, necessitating long chemical determinations, but the final decision rests upon whether the product, made either in the factory or in the laboratory, meets the flavor approval of a quality committee (5). Some rapid method such as the determination of the extinction values or the  $\alpha$ -dicarbonyl content of the volatile essential oil might be correlated with the flavor examination of the finished product, thereby giving a rapid and accurate method for the estimation of the flavor value of the botanical.

At the present time extinction values and  $\alpha$ -dicarbonyl values are included in the specifications for the purchase of raw ma-

terials, for it is believed that they give some indication of the geographical growing district and the age of the botanical. Both the growing district and the age are very important from a flavor standpoint.

Volatile essential oils were obtained from botanicals, many of which were purchased during 1939 and had been in cold storage for periods varying from a few to 30 months. The essential oils were not commercially rectified but were recovered by the Clevenger method of steam-distillation (3). If the distillation is continued for at least 8 hours, the volatile essential oils obtained frequently have a yellow color, especially if the oil has been distilled from botanicals that have been in storage for some time. The color might be caused by  $\alpha$ -dicarbonyl compounds which impart color to autoxidized oils of animal and vegetable origin (4). The exact mechanism leading to the formation of colored substances is not fully understood, but there is agreement on the assumption that the yellowing is caused by diketone groups when fatty oils age. A simple test devised for the detection of  $\alpha$ -dicarbonyl compounds in fatty materials (4) has been extended to volatile essential oils.

## EXPERIMENTAL

**METHODS.** Physicochemical data on the oils, such as index of refraction, specific gravity, acid number, and ester number, were obtained by standard procedures (2).

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Transmittance measurements of the oils were made with a Coleman Universal spectrophotometer. The measurements would have been of greater significance in the ultraviolet range of the spectrum in which maxima and minima would have been more clearly defined. The undiluted oils were placed directly in transmission cells of 1-cm. diameter, and compared with a blank cell containing the same quantity of water. Extinction values ( $\log 1/T$ ) were read off directly from the drum scale of the instrument.

**REAGENTS.** Oximation solution, 3% hydroxylamine hydrochloride in pyridine. Solutions for forming the bis-pyridine-ferrous derivatives of dioximes: 60 grams of Rochelle salt plus 100 ml. of water; 5 grams of ferrous sulfate plus 100 ml. of water; 100 grams of potassium hydroxide plus 100 ml. of water.

**METHOD.** Pipet 0.5 gram of volatile oil into a test tube and oximate by adding 2 ml. of hydroxylamine hydrochloride solution. React for 2 hours in a water bath at 80° C.; cool, add 0.3 ml. of acetone to destroy the excess of hydroxylamine, and let stand for 5 minutes. The highly colored bis-pyridine-ferrous derivative of the dioxime is formed as follows: Add 1 ml. of Rochelle salt solution and 1 ml. of ferrous sulfate solution, then slowly add, with shaking, 4 ml. of potassium hydroxide solution. A positive test for  $\alpha$ -dicarbonyl compounds is the formation of an intense red color in the pyridine layer. A blank is run with reagents only.

The test was run on solutions containing known amounts of biacetyl and dimethylglyoxime; the preliminary oximation procedure is not necessary for the dimethylglyoxime. Solutions were made by weighing out 10, 20, 30, 40, and 50 millimoles, dissolving in alcohol, and diluting to 1 liter. The colors developed by the oils were visually compared with the colors developed by the known solutions. In this way a semiquantitative estimation of millimoles of  $\alpha$ -dicarbonyl compounds present in the original volatile oils could be made.

#### JUNIPER BERRY OIL

Extinction values were obtained using fifteen oils, steam-distilled from shipments of juniper berries, *Juniperus communis*, some of which had been used in the plant production of gin. Therefore the flavor values of some of the berries have been evaluated by actual use in production.

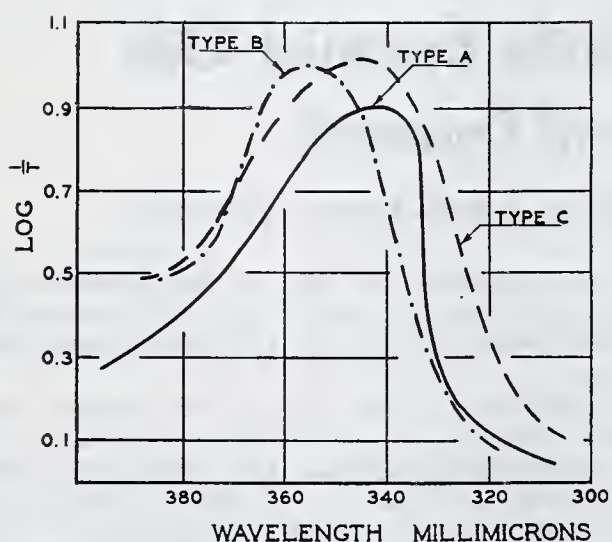


Figure 1. Absorption Curves of Juniper Berry Oil Types

Type A, oil of ordinary Italian berries  
Type B, oil of select Italian berries  
Type C, oil of domestic berries

Figure 1 shows that juniper berry oils obtained from berries which had been in cold storage for 25 to 30 months give three distinct curves.

Type A with a maximum extinction value at 340 mμ represents oils distilled from ordinary Italian berries scooped from the ground and bushes; type B with a maximum extinction at 355 to 360 mμ represents oils distilled from berries which were hand-picked and selected in Italy; type C with a maximum extinction at 345 mμ represents oil distilled from berries grown in eastern United States. The berries which produced the finest flavored gins when used in the factory contained oils which gave a maximum extinction at 355 to 360 mμ, such curves rising rapidly and

Table I. Aging of Juniper Berry Oils within the Berry and in Soft-Glass Bottles

Oil No.	Description	Extinction Values at Following Wave Lengths					
		320 mμ	340 mμ	350 mμ	360 mμ	370 mμ	380 mμ
1	Freshly distilled from berries	0.18	0.90	1.20	1.10	0.92	0.74
2	Oil 1 aged 60 days in soft glass	0.20	0.94	1.18	1.20	1.05	0.72
3	Freshly distilled from berries stored in cold for 30 months	0.18	1.06	1.05	0.85	0.67	0.54
4	Freshly distilled from same berries stored in cold for 46 months	0.60	1.10	1.22	1.22	1.00	0.84

falling almost as soon as the maximum extinction is reached the whole shape of the curve is almost as important as the maximum extinction value. Berries whose oils give extinction values similar to type A are inferior in quality, and berries whose oils give extinction values similar to type C are very much inferior from a flavor standpoint. Therefore, the extinction values of an oil distilled from an unidentified source of juniper berries will give information as to the flavor value of such berries.

A commercial juniper oil, Juniperol (Fritzsche Bros.), distilled between 160° and 200° C. in the laboratory gave a curve much like type A oils except the maximum extinction value was 0.5 instead of 0.9.

Most of the volatile oils deteriorate rapidly unless kept in small, filled, tightly stoppered bottles in a cool, dark place. If exposed to air, light, and warmth they resinify, and the oxidized resinous products formed change the volatile oils in composition, color, specific gravity, and odor. There is spectroscopic evidence that aging does occur in the botanical during cold storage for 25 to 30 months, but at a much less rapid rate than during an additional 30 months' storage. Aging in the botanical during cold storage for the next 15 months after 30 months' storage is very rapid and almost equal to aging of the oil outside the botanical.

The steam-distilled juniper berry oils were stored in soft glass bottles for 60 days at room temperature on top of the laboratory table away from the sunlight. An example of the change due to such accelerated aging can be demonstrated by spectral data.

Oil 1, Table I, shows the extinction values for freshly distilled juniper oil from the berries, and oil 2 shows the extinction value for the same oil after accelerated aging. Aging of the juniper oils in every case shifts the extinction maximum toward the light of higher wave lengths, and the near maximum is maintained over a greater wave-length span. The extinction value in the higher wave lengths do not fall as rapidly as those of the fresh oil. Some of the juniper oils show more resistance to accelerated aging than others, and in most cases the extinction values increase greatly in the higher wave lengths. Oils stored in the dark at icebox temperatures show no change in the spectral data.

Oil 3, Table I, shows the spectral extinction values of a freshly distilled juniper oil taken from berries which had been stored at refrigerator temperatures for 30 months. Oil 4, Table I, shows the spectral extinction values of the freshly distilled oil taken from the same berries after cold storage for 46 months. There has been rapid aging of the oil in the berries between 30 and 46 months of storage. Extinction values will give some evidence of the aging of oil in the berries, and such data will make it possible to purchase fresh stocks of raw materials and maintain a high flavor level.

Table II shows the change in some of the chemical constants of the juniper oils after aging. The index of refraction, specific gravity, acid number, and ester number increase rapidly when the oil is stored in soft-glass bottles placed on the laboratory desk top at room temperature. Oil distilled from domestic juniper berries shows a great increase in density and ester number when aged outside the berry. Resinification was very noticeable when the domestic berry oil was aged outside the berry, but there was little change in the oils steam-distilled from the Italian berries



**Table II. Chemical Constants of Steam-Distilled Juniper Oils**

(Before and after aging in soft-glass bottles at laboratory temperatures and room light)

Source of Oil	Index of Refraction, 20° C.	Specific Gravity, 25° C./25° C.	Acid No.	Ester No.
Ordinary eastern United States berries				
Fresh oil	1.4780	0.8530	1.05	8.98
Aged oil	1.4880	0.8815	1.91	11.77
Ordinary Italian berries				
Fresh oil	1.4800	0.8725	1.71	6.91
Aged oil	1.4830	0.8807	2.23	8.31
Italian hand-picked and selected berries				
Fresh oil	1.4812	0.8626	1.15	5.25
Aged oil	1.4825	0.8775	1.92	5.15

**Table III. Chemical Constants and  $\alpha$ -Dicarbonyl Content of Juniper Oils**

Sample No.	$n_D^{20}$	Acid No.	Ester No.	Millimoles of $\alpha$ -Dicarbonyl Compounds per Kg. of Volatile Oil
1	1.4782	0.98	3.4	0
2	1.4760	0.83	7.4	0
3	1.4811	0.83	7.4	0
4	1.4788	1.07	8.1	20
5	1.4788	1.07	6.8	20
6	1.4792	1.07	6.3	30
7	1.4812	2.50	9.7	50
8	1.4828	2.90	11.9	50
9	1.4790	9.10	18.1	90
10	1.4802	7.00	20.6	90

All the aged oils had a turpentinelike odor. The juniper berry oil aged in the berry and stored in cold storage between 30 and 46 months showed increases in acid and ester number.

Table III shows the chemical constants and  $\alpha$ -dicarbonyl content of various steam-distilled juniper berry oils.

Oils 1, 2, and 3 were distilled from hand-picked and selected Italian juniper berries stored for 30 months in cold storage. No  $\alpha$ -dicarbonyl compounds could be detected in these volatile oils. Oils 4, 5, and 6 were distilled from Italian berries stored for 46 months in cold storage. Oils 7 to 11 were distilled from juniper berries that had been stored in sealed tin containers at room temperature for 5 years, berries 7 and 8 were select Italian berries,

and berries 9 and 10 were ordinary German juniper berries. The increase in  $\alpha$ -dicarbonyl content is in direct correlation with the age of the berries.

Unsaturated carbonyl compounds show bands of strong absorption at low wave lengths, but absorption in the higher wave lengths, such as used for the experimental work of this paper, are said to be due to conjugated systems. The intensive absorption bands are displaced to longer wave lengths with increasing length of the chromophoric conjugated systems. The atmospheric distillation of aged commercial juniper oil showed that the compounds responsible for the shift in maximum extinction value could be concentrated in the residue. Treatment of the aged commercial juniper oil with activated carbon changed the extinction values slightly, but it was not possible to rejuvenate the aged oil by carbon treatment. Acetylation of a very pure fraction of commercial juniper oil moved the extinction maximum from 340 to 370  $m\mu$  and gave values similar to aged juniper oil.

Table IV shows the  $\alpha$ -dicarbonyl content, acetyl number, and months in cold storage for a number of juniper oils. The acetyl number can be used as a method of differentiation between domestic and select Italian berries. There was definite positive correlation between the  $\alpha$ -dicarbonyl content and the acetyl number of the juniper oils.

**Table IV. Comparison of  $\alpha$ -Dicarbonyl Content and Acetyl Number of Freshly Distilled Juniper Oils**

Identification	Months in Cold Storage	Relative $\alpha$ -Dicarbonyl Content	Acetyl No.
Select Italian	46	2	41.9
Select Italian	46	1	45.3
Select Italian	45	3	43.6
Ordinary Italian	46	3	70.4
Ordinary Italian	46	3	76.8
Select Italian	42	2	52.3
Select Italian	39	0	35.2
Ordinary United States	17	3	106.0

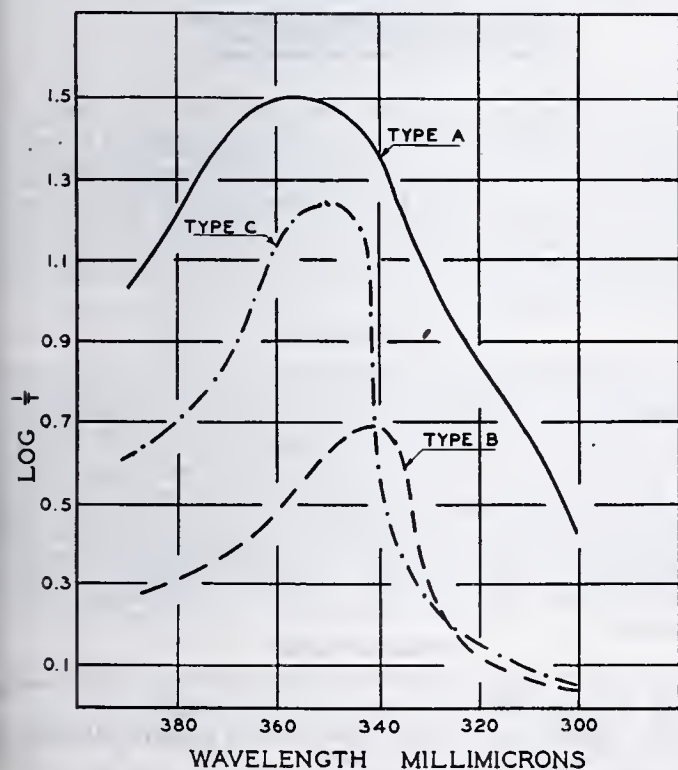
#### ORANGE PEEL OIL

Orange peel, bitter and sweet, is used in the manufacture of cordials and liqueurs. Bitter orange oil differs very slightly from sweet orange oil physically and chemically; hence specifications for the purchase of orange peel do not always help to select the peel with the correct flavor character. Extinction values and the odor of the acetylated oil freshly distilled from an unknown peel will be helpful in identifying true bitter orange peel.

Extinction values were obtained using 15 orange oils steam-distilled from shipments of bitter peel, imported sweet quarter peel, and ribbon peel of sweet oranges grown in the United States. Any single oil examined gave one of the three types of absorption curves shown by Figure 2. Oils steam-distilled from bitter peel showed maximum extinction at 355  $m\mu$  (type A), oils steam-distilled from domestic sweet orange ribbon and Haitian ribbon peel showed maximum extinction at 340  $m\mu$  (type B), and oils steam-distilled from Sicilian sweet orange peel quarters showed maximum extinction at 350  $m\mu$  (curve C). Practical plant use of the many sweet orange peels used in this study demonstrated that Florida sweet ribbon peel can be used in place of Haitian ribbon peel for sweet orange distillates; both peels have the same spectral identity.

The steam-distilled orange oils were stored in soft-glass bottles, partially filled, on the laboratory table at room temperature. The oils were very prone to oxidation, and quickly deteriorated in flavor value during such storage conditions. Table V, oil 1, shows the extinction values of a fresh oil distilled from Florida ribbon peel; oil 2 shows the extinction values for the same oil after accelerated aging for 60 days. Aging of the oil shifts the extinction maximum towards the higher wave lengths and greatly increases the optical density at its maximum (1.5 instead of 0.5 extinction).

Orange oils freshly distilled by the Clevenger method, ir-

**Figure 2. Absorption Curves of Orange Peel Oil Types**

Type A, oil of bitter orange peel  
 Type B, oil of Florida ribbon orange peel  
 Type C, oil of Sicilian sweet orange peel quarters



Table V. Aging of Sweet Orange Peel Oil and Effect of Adding Ethyl Anthranilate and Decyl Aldehyde to Sweet Orange Oil

Oil No.	Description	Extinction Values at Following Wave Lengths					
		320 m $\mu$	340 m $\mu$	350 m $\mu$	360 m $\mu$	370 m $\mu$	380 m $\mu$
1	Freshly distilled from Florida ribbon peel	0.10	0.52	0.46	0.35	0.31	0.26
2	Oil 1 aged 60 days in soft glass	0.10	1.03	1.25	1.44	1.50	1.43
3	Commercial sweet orange oil, fraction distilled at 174–177° C.	0.20	0.58	0.58	0.50	0.37	0.28
4	Addition of ethyl anthranilate to oil 3	0.30	0.90	1.13	1.27	1.12	0.90
5	Addition of decyl aldehyde to oil 3	0.20	0.58	0.58	0.50	0.39	0.31

Table VI. Chemical Constants of Steam-Distilled Sweet Orange Oils

(Before and after aging in soft-glass bottles at laboratory temperatures and room light)

Source of Oil	Specific Gravity, 25° C./25° C.	Ester No.	Acid No.
Florida ribbon peel			
Fresh oil	0.8403	2.30	0.7
Aged oil	0.9242	44.62	5.5
Sicilian peel in quarters			
Fresh oil	0.8420	4.30	0.8
Aged oil	0.9342	51.30	8.7

respective of the age of the peel or the storage conditions, gave negative tests for  $\alpha$ -dicarbonyl compounds. Even after the accelerated aging period of 60 days many of the oils contained less than 10 millimoles of  $\alpha$ -dicarbonyl compounds per kg. of oil. It was noticed that oils distilled from peels with a very small amount of pulp developed a higher  $\alpha$ -dicarbonyl content during the aging period of 60 days. Perhaps there is an antioxidant in the pulp of the peel.

Table VI, chemical constants of fresh and aged sweet orange oils, shows a great increase in ester number for each aged oil.

In order to understand better what chemical change is responsible for the shift in extinction maxima of sweet orange oils during aging, ethyl anthranilate (Felton Chemical Co.) and decyl aldehyde (Florasynth Laboratories), both freshly distilled at atmospheric pressure, were added to the fraction (b.p. 174–177° C.) of oil of sweet orange (Fritzsche Brothers, U.S.P.) whose extinction values are shown as oil 3, Table V. When 0.5 ml. of ethyl anthranilate was added to 10 ml. of oil 3, the extinction maximum shifted towards the visible light, oil 4, Table V. When 0.1 ml. of decyl aldehyde was added to 10 ml. of oil 3, the extinction values remained unchanged (oil 5, Table V). None of the individual ester, aldehyde, or oil had a visual yellow color.

Atmospheric distillation of commercial bitter orange oil into two fractions and residue showed that the compounds responsible for the extinction maximum shift of aged oil can be concentrated in the residue just as was done with juniper oil. The extinction values of the first fraction (b.p. 175–177° C.) were identical to those of a good sweet orange oil. The second fraction (b.p. 177–185° C.) gave extinction values characteristic of good bitter orange oil. The spectral data that enable one to differentiate between bitter and sweet oil and thereby ensure good flavor are due to fraction 2 and the residue. Over one half of the bitter orange oil is the same chemically when measured by spectral data as sweet orange oil.

Acetylation of all the fractions of bitter orange oil caused the formation of a yellow color or intensification of the yellow color. During the course of acetylation it was very noticeable that all fractions of acetylated bitter orange oil had a foreign, characteristic odor. Fraction 1 had an acetyl number of 11.9, fraction 2 had an acetyl number of 21.4, and the residue had an acetyl number of 50.1. The characteristic odor formed by acetylation will give an additional test to differentiate bitter orange oil from sweet orange oil, thereby maintaining the correct balance of flavor in the manufacture of cordials and liqueurs.

The acetyl numbers of sweet orange oils steam-distilled from orange peels which have been stored for about 40 months were

lower than fraction 1 of the bitter orange oil. This is additional evidence of antioxidants in the peel.

#### CORIANDER SEED OIL

The fruit of the *Coriandrum sativum*, known as coriander seed, is used as an ingredient in the manufacture of gin. Seeds have been offered for sale from at least nine geographical growing districts of Europe and Africa. Ultraviolet absorption studies have been made of the alcoholic solutions of the various essential oils obtained by the Clevenger steam-distillation of the seeds. The extinction values showed that the distilled oils from seeds grown in different geographical districts could be differentiated from one another by their absorption curves (1).

Extinction values were obtained using 5 coriander oils distilled from shipments designated as seeds grown in England, Hungary, and Russia. The absorption curves for Hungarian seed oil (type A), English seed oil (type B), and Russian seed oil (type C), all of Figure 3, show a great likeness between the Russian and Hungarian oils; the English oil is different. Russian and Hungarian seeds are more desirable from a flavor standpoint for the manufacture of gin than are seeds of English origin.

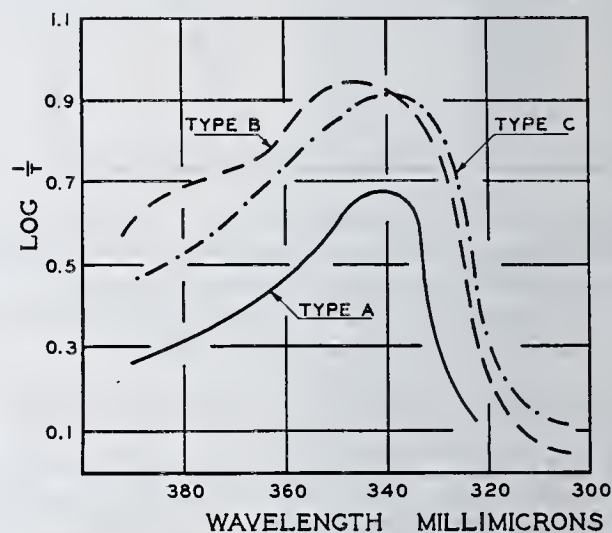


Figure 3. Absorption Curves of Coriander Seed Oil Types

Type A, oil of Hungarian coriander seed  
Type B, oil of English coriander seed  
Type C, oil of Russian coriander seed

Accelerated aging of Russian coriander seed oil caused the same type of extinction shift as reported for juniper berry oil and orange peel oil. Even though the oils steam-distilled from seeds grown in England and Russia differ, their absorption curves after aging are almost identical. The oil of the coriander seed remains fresh within the seed when stored under ideal conditions for at least 40 months.

#### CONCLUSION

The distiller must have information at hand that assures him the same type of botanical is being used in each successive distillation if uniformity is to be maintained, for it is true that the same berry, bark, or seed from even slightly different geographical areas produces beverages of different flavor. The nature and composition of the oils, in addition to the quantities which exist in the botanicals, are responsible for the variations in flavor-imparting value. Spectral data assist in maintaining correct flavor value.

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# Use of Iodine Monochloride in Standardization of Permanganate Solutions with Arsenious Oxide

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Permanganate solutions can be standardized against arsenious oxide by using iodine monochloride as a catalyst and *o*-phenanthroline ferrous complex as indicator. The end point is attained rapidly, is stable, and no correction for the catalyst is required regardless of the amount added. Results obtained agree with values obtained with oxalate by the method of Fowler and Bright to within 1 part in 3000.

EXPERIMENTAL studies by Kolthoff, Laitinen, and Lingane (5) and Bright (1) have confirmed the observations made by Lang (6) and subsequently by others (2, 7-9) that arsenious oxide can be used as a satisfactory primary standard for permanganate solutions provided an iodine compound is added to the titrated solution. Kolthoff and co-workers (5) and Bright (1) made use of both potentiometric and visual end points; the visual end points were obtained both by the permanganate color and by using *o*-phenanthroline (1,10-phenanthroline)-ferrous sulfate complex as an internal potential indicator.

Potassium iodate was used by Kolthoff and his co-workers, and either potassium iodide or iodate by Bright; in all cases 1 drop of a 0.0025 formal<sup>1</sup> solution of the iodine compound was used in final volumes of from 120 to 175 ml. Upon making titrations under the conditions outlined by the above workers, and using visual end points, it was evident that the rate of the titration reaction became slow in its final stages. Even 2 ml. before the end point, when using approximately 0.02 formal (0.1 *N*) permanganate solutions, the pink color of the *o*-phenanthroline indicator faded upon addition of each drop of permanganate and 5 to 10 seconds were required for the original color to return; closer to the end point this effect became much more pronounced. As a result, considerable time and patience are required to establish the correct end point, and persons not familiar with the end point are likely to obtain results significantly in error. It seemed probable that this difficulty might be minimized if a larger amount of the catalytic iodine compound were added; also, if at the end point substantially all the iodine were present in a single oxidation state, it would be possible to add a compound in which the iodine had this oxidation state and thus avoid the necessity of a correction for the catalyst regardless of the amount added.

Because of the stability of the end point in hydrochloric acid solutions the use of the *o*-phenanthroline indicator seemed desirable, and preliminary potentiometric titrations indicated that at the end-point color of this indicator substantially all the iodine was in the unipositive state. This conclusion was confirmed by titrating arsenious acid solutions to the *o*-phenanthroline end point and then adding relatively large amounts of an iodine monochloride solution without causing a significant change in the end point.

There are presented below the results of experiments which were made in order to determine the conditions under which arsenious oxide can be used as a primary standard for permanganate solutions when iodine monochloride is used as a catalyst and *o*-phenanthroline ferrous sulfate as the indicator.

## PREPARATION OF CHEMICALS AND SOLUTIONS

An approximately 0.02 formal solution of potassium permanganate was prepared and allowed to age for 2 months, then

Formal concentrations, formula weights per liter of solution, have been used because of the uncertainty attached to the use of normal concentrations with such compounds as potassium iodate and iodine monochloride which may have various changes of oxidation state.

filtered through a glass filter, and stored in an all-glass light-protected bottle. A 0.002 formal solution of potassium permanganate was prepared daily by accurately diluting the 0.02 formal solution.

Bureau of Standards sodium oxalate No. 40c was used, and the purity taken as 99.95%. Bureau of Standards arsenious oxide No. 83 was also used, and the purity taken as 99.98%. Each was dried for 1 hour at 105° C. immediately before use.

An iodine monochloride solution was prepared by the reaction of potassium iodide and iodate in 4 formal hydrochloric acid, using the disappearance of the carbon tetrachloride-iodine color to determine the end point; it was standardized with sodium thiosulfate. A 0.0025 formal solution was prepared by diluting the above solution with 2 or 4 formal hydrochloric acid.

Six formal sodium hydroxide was prepared and stored in a glass bottle provided with a paraffin-coated glass stopper. Rubber-stoppered bottles were found unsatisfactory for the storage of sodium hydroxide solutions which are to be used in oxidimetry.

Approximately 0.05 formal sodium oxalate solutions were gravimetrically prepared in 1.8 formal sulfuric acid (4). These solutions were used for no longer than 2 days.

Approximately 0.05 formal arsenious acid solutions were prepared by dissolving the weighed arsenious oxide in 25 ml. of 6 formal sodium hydroxide, adding 63 ml. of 12 formal hydrochloric acid and 2.7 grams of sodium chloride, and diluting to 500 grams.

The potential indicator was 0.025 formal *o*-phenanthroline ferrous sulfate as obtained from the G. Frederick Smith Company.

## PRELIMINARY EXPERIMENTS

A series of titrations was made using the *o*-phenanthroline indicator procedure of Bright (3) except that successively increasing volumes of the 0.0025 formal iodine monochloride solution were added. With 0.25 ml. of the catalyst, the pink color of the indicator faded temporarily to a perceptible violet color upon the addition of a drop of 0.02 formal permanganate approximately 0.2 ml. before the end point; with 0.5 and 1 ml. of catalyst only a transient fading of the pink color was observed; and with 5 ml. of catalyst fading was observed when about 0.1 ml. from the end point. The first end point (a very pale blue color) was transient, and the pink color returned more slowly, and an additional drop of permanganate (0.02 *F*) was required. The resulting end point was usually stable. The return of the end point with 5 ml. of catalyst is attributed to a slow rate of oxidation of elementary iodine by permanganate under the conditions of the titration.

The characteristics of the end point when using in one case 0.04 ml. of 0.0025 formal potassium iodate—the amount of catalyst used previously (1, 5)—and in the other case 1.0 ml. of 0.0025 formal iodine monochloride were compared by titrating 2-ml. portions of 0.075 formal arsenious acid. The behavior of the indicator with the successive additions of permanganate is shown in Table I.

Table I. Titration Characteristics with Varying Amounts of Iodine Catalyst

(Volume: approximately 100 ml. of solution 0.5 *F* in HCl and 0.5 *F* in NaCl. Indicator: 0.04 ml. of 0.025 *F* *o*-phenanthroline ferrous sulfate. Catalyst: 0.04 ml. of 0.0025 *F* KIO<sub>3</sub> or 1 ml. of 0.0025 *F* ICl)

KMnO <sub>4</sub> Added, Ml.	Increment	Total	Observations	
			KIO <sub>3</sub> catalyst	ICl catalyst
0.04	0.04		Solution almost colorless	Pink faded slightly
0.08	0.12		Transient end point	Pink faded slightly
0.50	1.00		End point for 2 seconds	Pink faded slightly
0.50	1.50		End point for 2 seconds	End point for 0.5 second
0.50	2.00		End point for 3 seconds	End point for 0.5 second
0.25	2.75		End point for 3 seconds	End point for 0.5 second
0.04	3.14		End point for 1 second	Pink color faded slightly
0.04	3.18		End point for 4 seconds	Pink color faded slightly
0.04	3.22		End point for 13 seconds	End point for 1 second
0.03	3.25			End points permanent



Table II. Standardization of a Permanganate Solution against Sodium Oxalate and Arsenious Oxide

(Two separate solutions of both oxalate and arsenious oxide were prepared and series of titrations made using weighted portions of each solution. The values shown are the weight normalities of the permanganate.)

	Using Sodium Oxalate		Using Arsenious Oxide	
	Solution A	Solution B	Solution C	Solution D
	0.10792	0.10788	0.10791	0.10793
	0.10789	0.10788	0.10793	0.10791
	0.10793	0.10790	0.10794	0.10794
	0.10792	0.10793	0.10794	.....
	0.10787	0.10791	.....	.....
Av.	0.10791	0.10790	0.10793	0.10793
Average deviation, %	0.019	0.015	0.009	0.009
Maximum deviation, %	0.06	0.05	0.03	0.03

A series of comparison titrations was then made, in order to check the precision of titrations made with the large amount of iodine monochloride and the agreement which could be obtained with the titer values obtained using sodium oxalate.

#### TITRATION PROCEDURES

**TITRATION OF ARSENIUS ACID SOLUTION WITH PERMANGANATE.** Approximately 50 grams of the arsenious acid solution were weighed out into a 400-ml. beaker and diluted to 100 ml. with water (giving a solution 0.2 formal in sodium chloride and 0.6 formal in hydrochloric acid). One milliliter of 0.0025 formal iodine monochloride in 4 formal hydrochloric acid was added and the 0.02 formal potassium permanganate solution was added rapidly from a weight buret, with continuous stirring, until a lag in the decolorization of the permanganate was noticed. The permanganate was then added dropwise (still with continuous stirring) until the permanganate color spread almost throughout the solution. (The indicator is advantageously withheld until this preliminary indication is obtained, since it permits

rapid addition of permanganate; otherwise the pink color of the phenanthroline complex would prevent observation of the permanganate color.) There was then added 0.04 ml. of the indicator and the titration was continued dropwise with the 0.02 formal permanganate until the first transient fading of the indicator was detected; this is usually within 1 drop of the end point. The 0.002 formal permanganate was then added from a 10-ml. volumetric buret until the pink color could no longer be detected. A blank solution was prepared, the end point checked again, and the blank solution titrated. The blanks required from 0.15 to 0.2 ml. of the 0.002 formal permanganate.

**TITRATION OF SODIUM OXALATE WITH PERMANGANATE.** The procedure of Fowler and Bright (3) was followed in detail.

The results of the titrations are shown in Table II.

#### ACKNOWLEDGMENT

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## Determination of 2,3-Butylene Glycol in Fermentations

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**BROCKMANN** and **Werkman** (1) described a method for the determination of 2,3-butyleneglycol in fermentations, which was much more satisfactory than previous methods, but had a number of defects. Recoveries were low, averaging 96.4%. The distillation step, involving collection of 1 liter of distillate from 25 ml. of solution, was cumbersome. Moreover, if sugar was present in the sample, interfering substances were formed during the alkaline distillation. The sample required was large (20 mg. or more).

In the procedure described below, the butylene glycol is separated from the culture by continuous ether extraction. Periodate oxidation is used, as in the method of Brockmann and Werkman. The acetaldehyde is determined by titration of the sulfite liberated when the acetaldehyde-bisulfite complex is made alkaline, in a step practically identical with that used by Friedemann and Graesser (2) in lactic acid determination. Iodometric acetaldehyde titration has been applied to butylene glycol determination in urine by Westerfeld and Berg (5). Recoveries are high (99%) and reproducible. There is little interference from constituents of culture media. From 2 to 10 mg. of butylene glycol are required.

This procedure has been in use for more than 5 years, and has given reliable results in student laboratories.

#### EXTRACTION

Since 2,3-butyleneglycol is extracted relatively slowly from water by ether, continuous extraction is necessary for from 8 to 48 hours, depending on the design of the extraction apparatus. In the author's laboratory, small continuous extractors, holding 5 ml. of sample, are used in routine determinations. A battery of these, attached to a multiple condenser and heated by one

steam plate, are used to extract for 12 hours or more 5 ml. of culture or diluted culture, adjusted to pH 7 or higher (to prevent extraction of lactic acid, which interferes). The time necessary for complete extraction should be determined by experiment before a standard extraction time is selected. Dilution of the cultures before extraction is desirable if frothing difficulties are encountered. A large extractor is used with larger volumes of culture for extraction times of 24 to 72 hours.

After completion of the extraction, water is added to the ether extract, the ether is evaporated, and the aqueous sample is diluted to a known volume.

#### DETERMINATION

The apparatus used consists of a 300-ml. Kjeldahl flask fitted with a dropping funnel and a short glass condenser. A spray trap should be placed between the boiling flask and the condenser. Ground-glass connections are better than a rubber stopper, but if a stopper is used, it should be cleaned in boiling alkali before use. The tube at the delivery end of the condenser should be long enough to extend to the bottom of the receiver flask. The sample, containing 10 mg. or less of 2,3-butyleneglycol, is pipetted into the Kjeldahl flask, 5 ml. of approximately normal sulfuric acid are added, and sufficient water to bring the total volume to about 50 ml. A pinch of talc is added to prevent bumping. In the 250-ml. Erlenmeyer receiver flask are placed 10 ml. of sodium bisulfite solution (12.5 grams per liter), made from fresh reagent and smelling strongly of sulfur dioxide. The end of the condenser should dip below the level of the bisulfite solution.

The burner is lighted, and as soon as the vapors reach the condenser 25 ml. of 0.01 *M* potassium periodate (2.3 grams per liter) are added by slow dropping from the dropping funnel. The rate of addition should be such that 25 ml. are added during 4 or 5 minutes. If periodate addition is begun before boiling begins, the first acetaldehyde produced will be mixed with air, and may not be completely absorbed by the bisulfite. If periodate addition is



played long after distillation is begun, butylene glycol will be in the distillate. Distillation is continued for 5 minutes after addition of the periodate has been completed. During the last minute of the distillation, the receiver flask is lowered, so that the end of the condenser no longer extends below the surface of the distillate. When distillation is stopped, the bisulfite adhering to the end of the condenser is rinsed into the flask.

Iodine solution (0.2 *N* is a convenient strength) is now added to the flask to oxidize the excess bisulfite. The iodine is run in carefully as the end point is approached. A drop or two of excess iodine is added, and, after the addition of starch indicator, the solution is adjusted to an accurate end point by weak thiosulfate. The sample is now ready for titration of the bound bisulfite, according to the procedure of Friedemann and Graeser (2). About 10 ml. of saturated sodium bicarbonate are added, and standard 0.01 *N* iodine from a buret is added rapidly, preferably at a rate such that the found bisulfite is oxidized as rapidly as it is liberated. When bisulfite liberation slows down, 5 ml. of bicarbonate are added, and the titration is carried to completion. One milliliter of 10% sodium carbonate is then added to ensure complete bisulfite liberation.

One equivalent of iodine corresponds to 0.25 mole of butylene glycol.

A blank determination should be run, and should give a titration of about 0.1 ml. of 0.01 *N* iodine. This blank value is applied as a correction to each determination.

### RESULTS

As may be seen from Table I, recoveries by the oxidation and titration procedure average about 99%. The 2,3-butyleneglycol as a sample of the meso compound, three times recrystallized, has a melting range (thermometer immersed in sample, rate of temperature rise, 0.2° C. per hour) was from 34.1° to 34.2°.

In Table II, the recoveries obtained in the extraction step are given. Each extractor (small vertical type) contained 5 ml. of 10% Difco yeast extract or 13% acid-hydrolyzed wheat mash. Each sample contained 110.2 mg. of glycol. The extraction time was 15 hours. Extraction from the clear medium was practically quantitative, but recovery from the hydrolyzed wheat mash, which contained a small amount of apparent glycol and much suspended material, was more erratic.

### CORRECTION FOR ACETON

Most cultures containing 2,3-butyleneglycol also contain acetoin, which will be extracted by ether. On periodate oxidation, it yields one molecule of acetaldehyde. When the acetoin content of the sample is determined by an independent method, a

Table III. Determination of 2,3-Butylene Glycol by Direct Titration

Glycol in Sample Mg.	pH During Oxidation	Glycol Found Mg.	Recovery %
4.208	a	4.172	99.3
		4.164	99.1
3.386	a	3.397	100.3
		3.396	100.3
1.693	a	1.712	101.1
		1.707	100.8
2.617 <sup>b</sup>	a	2.594	99.2
		2.594	99.2
3.386	a	3.390	100.1
		3.388	100.1
3.386	2.6	3.400	100.4
3.386	4.8	3.402	100.4
3.386	7.0	3.900	115.0
	7.0	3.862	113.9
1.693	7.0	2.393	141.2
	7.0	2.443	144.2

a 0.067 *N* H<sub>2</sub>SO<sub>4</sub>.

b Sample consisted of acetoin, recovery calculated as acetoin.

correction may be made for the interference caused. The acetoin method used in this laboratory is a modification of the method of Langlykke and Peterson (3). The foaming often encountered during the distillation in this method is obviated by dilution of the culture before distillation. To 10 ml. or less of distillate are added 2 ml. of *N* sodium hydroxide and 5 ml. of 0.02 *N* iodine. After 10 minutes, the sample is acidified and titrated with 0.005 *N* thiosulfate.

### DIRECT TITRATION OF 2,3-BUTYLENE GLYCOL

In the absence of other compounds oxidizable by periodate, 2,3-butyleneglycol may be determined by titration of the excess periodate.

The sample, 10 ml. or less in volume, containing not more than 4 mg. of glycol, is pipetted into a 25 × 200 mm. test tube, 1 ml. of 1.0 *N* sulfuric acid and 5 ml. of potassium periodate solution (2.3 grams per liter) are added, and the contents of the tube are mixed. The tube is then heated for 10 minutes in a boiling water bath. After cooling, 5 ml. of 0.5 *M* of sodium dihydrogen phosphate solution are added. The contents of the tube are well mixed, and 1 ml. of potassium iodide solution (300 grams per liter) is added. The liberated iodine is titrated immediately with 0.005 *N* thiosulfate from a 25-ml. buret. The weak thiosulfate will keep for only a few hours, and is therefore made as needed by suitable dilution of stronger standard thiosulfate. The difference in titration between a tube containing the sample and a blank tube is a measure of the butylene glycol content of the sample. One milliliter of 0.005 *N* thiosulfate is equivalent to 0.2253 mg. of 2,3-butyleneglycol. Acetoin interferes quantitatively, 1 mole of acetoin being equivalent to 1 mole of glycol.

In this procedure, the oxidation takes place in acid solution, but during the titration the pH is near 6.8. Under these conditions, periodate is reduced only to iodate, and large back-titrations are avoided. The titration of periodate in neutral solution has been applied to glycerol determination by Voris, Ellis, and Maynard (4).

From Table III, it may be seen that recoveries of slightly more than 100% are obtained with small samples, and that the presence of acid during the oxidation is essential. In the samples oxidized at definite pH values, the pH was held constant by suitable buffers. The titration step was always carried out at pH 6.8.

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Table I. Recovery of Pure 2,3-Butylene Glycol

Samples containing known amounts of pure butylene glycol were subjected to oxidation and titrated. They were not extracted with ether.

Glycol in Sample Mg.	Glycol Recovered Mg.	Recovery %
8.577	8.52	99.4
	8.47	98.8
4.233	4.180	98.8
	4.180	98.8
	4.203	99.3
	4.175	98.6
1.704	1.698	99.7
	1.698	99.7

Table II. Recovery of Butylene Glycol by Ether Extraction

Extractor No.	From yeast extract %	Glycol Extracted From hydrolyzed wheat <sup>a</sup> %
1	99.8	96.0
	99.4	96.2
2	99.5	99.2
	99.5	99.2
3	99.8	96.5
	99.8	97.2
4	98.2	....
	97.0	....
Blank (no glycol added to medium)	0.0	1.0
	0.1	1.0

<sup>a</sup> Corrected for apparent glycol in hydrolyzate.



# Determination of Moisture in Whole Egg Powder

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A method of determining the moisture content of whole egg powder with a standard error in precision of 0.01% is presented. The equipment required is generally available, or can be easily made.

**D**URING a study of the drying of egg powder to low moisture levels (less than 1%), the need for a fast and reproducible moisture determination method soon became evident. The A.O.A.C. method (2) is relatively slow, requiring 5 to 6 hours' oven time, and it is impossible to reach a constant weight. The apparent moisture determined by this method increases at a rate of 0.06% per hour of oven time after 4 hours' initial heating.

It has been shown by Cleland and Fetzer (3) that correct preparation of the sample will allow moisture contents of thermal-sensitive products to be determined by the distillation method. In order to obtain satisfactory results by this method with dehydrated products at low moisture levels, the sample weight becomes excessive. The procedure of Makower and Myers (4) whereby the vapor pressure of the foodstuff is determined leads to a fair degree of reproducibility, but the results have to be converted by comparison with some direct method if they are to be recorded as per cent moisture. The determination of low moisture levels apparently requires the use of elevated sample temperatures, thereby introducing the factor of possible decomposition of the product. The Fischer titration method (1) has been reported (personal communication) to give excellent results on egg powder, but requires the services of an experienced analyst, since frequent standardization of reagents is necessary. A sensitive potentiometer with low current consumption is also required.

Previous experience in the drying of degraded starch products had indicated the value of a drying procedure utilizing a high-vacuum chamber with good heat transfer to the sample being dried. The apparatus shown in Figure 1 was devised and tests were run to determine the time and temperature required to obtain consistent results with a minimum of decomposition as indicated by a small continuous change in apparent moisture.

## DESCRIPTION OF METHOD

The drying oven was made of two brass tubes of dimensions shown (Figure 1), all seams being silver-soldered and tested for leaks. It was considered advisable to use standard glass-stoppered weighing bottles, since these can be tightly closed to prevent moisture pickup while handling and weighing, are convenient to handle, and are light in weight. Outside ground cap weighing bottles 25 mm. in inside diameter and 50 mm. high were chosen, since a 3-gram sample of egg powder (apparent density 0.24 gram per cc., 15 pounds per cubic foot) could be easily placed in each bottle. These bottles had a cross-sectional area of 4.84 sq. cm. (0.75 square inch) and were high enough to prevent loss of sample when the pressure in the apparatus was suddenly reduced.

The holder illustrated in Figure 1, 10, maintained the bottles in a fixed position and was convenient to handle. It also allowed a large cross-sectional area to be maintained between the dry ice trap and the samples.

The top of the inner tube of the drying oven was connected to the dry ice trap using Pyrex tubing 17 mm. in outside diameter. Care was taken to maintain the full cross-sectional area of this tube at the bends. The connecting tubing was fastened to the U-tube by a short length of heavy walled rubber tubing, 1.25 cm. (0.5 inch) in inside diameter.

A standard 0.94-liter (1-quart) silvered Dewar flask held a mixture of methanol and dry ice. An excess of dry ice was added and the flask once filled needed no further attention for 8 hours or more. The U-tube trap was constructed of 17-mm. Pyrex tubing. It was found that this simple design of trap was stronger and just as effective as the more elaborate types. It

was easy to clean. It did not plug up at the bottom, since incoming condensable vapors collected along the vertical arm above the bend, almost all condensation occurring in the incoming arm of the trap. The trap could be inspected while in operation, since the frozen moisture formed an easily observable layer in the tube and the thickness of the collected layer could be estimated by looking down through the bend in the connecting tube. When after several determinations, the layer of condensables reduced the internal diameter of the U-tube to about 0.6 cm. (0.25 inch), the trap was removed, the ice melted by holding under running water, and the trap dried with a small quantity of acetone. The dry ice-methanol mixture provided a convenient method of maintaining a low vapor pressure, since dry ice is now available from almost all ice cream manufacturers.

The system was connected to a Cenco Hyvac pump which was allowed to run continuously during a determination. A McLeod gage was used to check the pressure on the pump side of the dry ice trap. Without special precautions it was possible to maintain a pressure of 0.05 mm. of mercury consistently, and all determinations were made at this pressure.

The jacket of the drying oven contained a small quantity of water and was maintained at the boiling point by a small gas flame. The condenser at the top returned the condensate to the jacket. Water losses were found to be negligible. Other liquids could be substituted for water if desired to maintain higher lower temperatures.

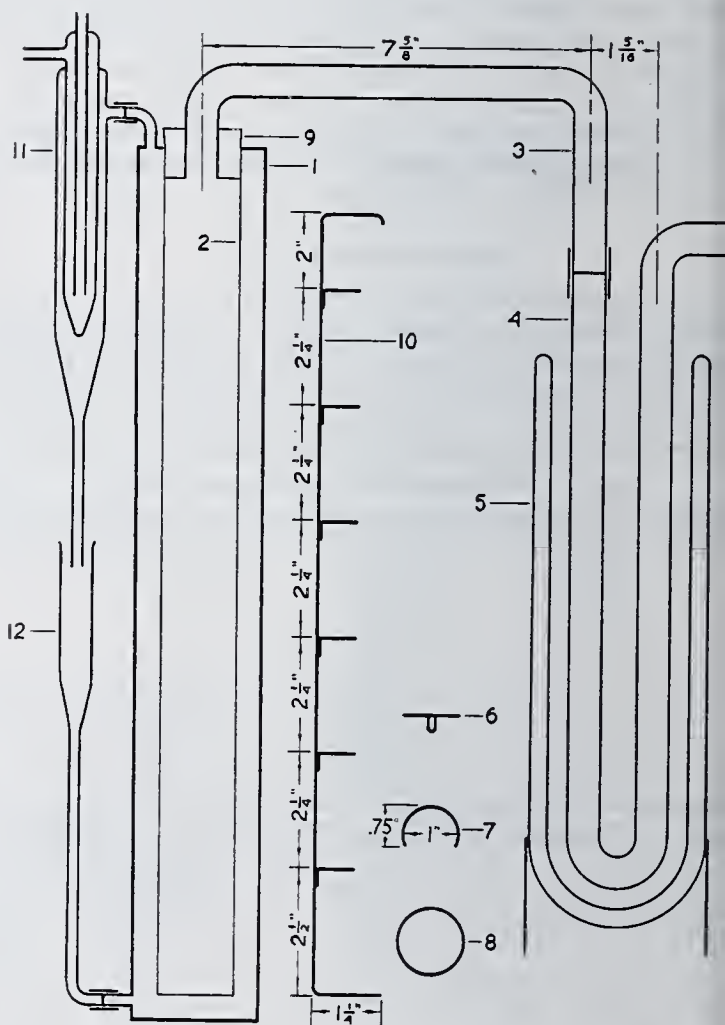


Figure 1. Apparatus for Determination of Moisture

(1) 17 X 2.5 inch O.D. 16-gage brass tube. (2) 16.5 X 1.5 inch O.D. 17 inch I.D. brass tube. (3) Connection tube, 17-mm. O.D. Pyrex tubing. (4) U-tube trap, 17-mm. O.D. Pyrex tubing, over-all length 12.375 inches. (5) Quart wide-mouthed Dewar flask. (6) Side view of bottle holder rings, brass wire 18-gage. (7) Top view of bottle holder rings. (8) Top view of base bottle rack. (9) No. 8 rubber stopper. (10) Bottle rack 14-gage brass wire with rings soldered. (11) Condenser. (12) Level indicator and condenser return



## EXPERIMENTAL PROCEDURE

Wash the weighing bottles, dry to constant weight ( $\pm 0.0001$  gm), and store in a desiccator containing anhydrous calcium sulfate. Place a  $3.0 \pm 0.5$ -gram sample of powder in the bottle, quickly stopper, and weigh to 0.0001 gram. When working with egg powder of less than 1% moisture, the time of exposure of the egg powder to the atmosphere must be less than 10 seconds. A cork borer 15 mm. in diameter may be used to collect the sample. Push the borer to a depth of 30 to 40 mm. into the powder to be sampled, withdraw carefully, hold above the weighing bottle, and tap gently to cause the plug of powder to fall into the bottle. A minimum area of powder surface is exposed to the atmosphere by this procedure. The sampling time may be held to 10 seconds without undue difficulty.

Place from 1 to 6 samples in the drying oven rack after removing stoppers. Place stoppers in desiccator and place samples in the hot oven. Connect the dry ice trap, start pump, and note time when pressure reaches 0.05 mm. of mercury (about 2 minutes after starting pump). Maintain water at gentle boil as indicated by condensate dripping from condenser and maintain vacuum for 75 minutes. Release vacuum through the dry ice trap over a period of 30 seconds, so that moist air will not be sucked into drying oven. It is advisable to restrict the vacuum release with a length of fine capillary tubing to ensure a slow entry of air through the dry ice trap to the drying oven. Disconnect trap, remove samples, stopper immediately, and place in desiccator. Allow to stand 20 minutes, or until cold, and weigh to 0.0001 gram. Empty weighing bottles, wipe thoroughly with an absorbent gauze, reweigh, and store in desiccator.

## SAMPLE PREPARATION

The results listed in Table I were obtained from three samples prepared as follows:

Samples of egg powder (300 grams) were sifted on a large sheet of paper, thoroughly mixed, resifted, mixed again, then placed in a 1-liter rubber-stoppered bottle. Six samples were run under each set of conditions. The time of treatment was taken from the time at which the pressure reached 0.05 mm. of mercury out 2 minutes after starting the pump.

Runs 1, 2, and 3 were made at 100° C., runs 5 and 6 at 78° C. The A.O.A.C. procedure was used for run 4. The results are collected in Table I and plotted in Figure 2.

## DISCUSSION OF RESULTS

When weighings on 3-gram samples are made to 0.0001 gram, the precision of the moisture percentage is  $\pm 0.007$  unit. The

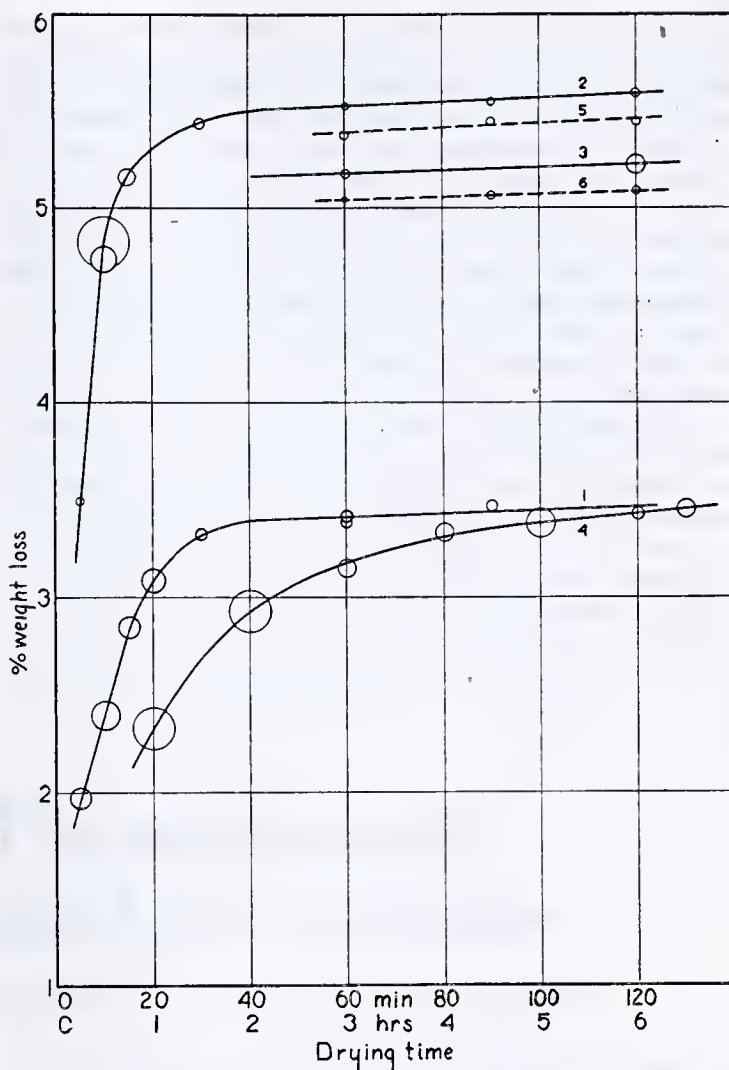


Figure 2. Moisture Determination

1 and 4. Fast method and A.O.A.C., respectively, run on same powder sample. 2 and 5. Fast method at 100° and 78° C., respectively, run on same powder sample. 3 and 6. Fast method at 100° and 78° C., respectively, run on same powder sample. Diameter of circles is four times standard error

difference between two weighings, each made to  $\pm 0.0001$  gram, is  $\pm 0.0002$  gram, and for a 5% moisture sample loss in weight is 0.1500 gram.

$$(0.0002/0.1500) \times 100 = 0.13\% \text{ error}$$

$$5 \pm (5 \times 0.13\%) = (5 \pm 0.007)\%, \text{ for 1 sample}$$

The standard error of the arithmetic mean for six samples would be:

$$\sqrt{\frac{\sum (x^2)}{N(N-1)}} = \sqrt{\frac{0.000294}{6 \times 5}} = 0.0031$$

for 5 samples, 0.0035, and for 2 samples, 0.007,  $x$  is the deviation of any determination from the arithmetic mean.  $N$  is the number of determinations.

The standard errors of the experimental results for 60, 90, and 120 minutes fall between 0.021 and 0.001, the mean being 0.008. The experimental error is about twice the calculated weighing error, showing that errors in handling, heating, and cooling the weighing bottles are small and of no significance. When handling egg powder of low initial moisture, the chance of moisture pickup will be increased, and greater variations of the result will occur unless careful technique is used. It was found that a 0.6-cm. (0.25-inch) layer of egg powder changed from 1.06 to 1.49% moisture in a 5-minute period when exposed to a 50% relative humidity 21.11° C. (70° F.) atmosphere.

The rate of decomposition at 100° C. in this fast method in terms of apparent moisture increase is 0.055% per hour, estimated from the best straight lines through the 60-, 90-, and 120-

Table I. Determination of Moisture

Run No.	Heating Time, Minutes	No. of Samples	Mean Weight Loss, %	Standard Error
1	5	6	1.969	0.023
	10	6	2.392	0.034
	15	6	2.846	0.025
	20	6	3.081	0.027
	30	6	3.318	0.009
	60	6	3.383	0.010
	60	6	3.412	0.015
	90	5	3.467	0.007
2	120	6	3.425	0.013
	5	5	3.490	0.007
	10	5	4.814	0.075
	10	6	4.737	0.029
	15	6	5.155	0.019
	30	5	5.434	0.011
	60	6	5.528	0.005
	90	6	5.549	0.008
3	120	6	5.593	0.008
	60	6	5.175	0.008
4	120	6	5.219	0.021
	60	3	2.327	0.050
5	120	3	2.929	0.050
	180	3	3.149	0.019
	240	3	3.333	0.020
	300	3	3.378	0.036
	390	3	3.453	0.021
	60	6	5.382	0.008
6	90	6	5.450	0.008
	120	6	5.451	0.006
6	60	6	5.041	0.001
	90	6	5.070	0.007
	120	6	5.088	0.005
	150	6	5.123	0.005



minute samples. In this laboratory a standard time of heating of 75 minutes is used. The maximum error from slow volatilization or decomposition of the samples in this period is 0.07. The rate of increase in apparent moisture appears to be reproducible to 0.02, and is of the same magnitude as found in the A.O.A.C. method. The end point of the determination is reached in 1.25 hours against 5 or 6 hours for the A.O.A.C., thus eliminating a large part of the uncertainty of the true end point. The break in the curve is very sharp compared to the A.O.A.C. curve, thus limiting the maximum time of decomposition to 75 minutes.

The runs made at 78° C., using ethanol in the heating jacket, show a rate of increase in apparent moisture slightly less than that found at 100° C.

The apparent moisture determined at 78° C. is 0.13% lower than that obtained at 100° C. It is probable that substances less volatile than water are driven off at the temperatures and pressures involved. The determination of the absolute value of the moisture content would then require an analysis of the vaporized materials at high vacuum and temperatures sufficiently low to avoid decomposition.

The determination made at 100° C. with a 75-minute heating period agrees closely with the A.O.A.C. method using a 5-hour

heating period; hence these conditions have been adopted standard in this laboratory. The precision of this fast method gives a reproducibility of 0.01 percentage unit, and the variables such as temperature, vacuum, and moisture pickup can be easily controlled.

For routine moisture determinations using duplicate sample weighings made to 0.001 gram will yield moisture percentage figures with a standard error of 0.07% moisture in the range 0.5 to 5% moisture. However, for determinations on powders with moisture contents under 1%, greater precision of weighing is indicated.

#### ACKNOWLEDGMENT

The assistance of S. S. Jarrett in determining moisture contents is gratefully acknowledged.

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## Determination of Total Sulfur in Feeds Modified Nitric and Perchloric Acid Digestion Procedure

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A method of determining the total sulfur content of feeds and similar substances by destroying the organic matter and oxidizing the sulfur compounds to sulfates by digestion with nitric and perchloric acids is described. The sample is dissolved and partially oxidized by heating with concentrated nitric acid on a steam bath for 24 hours. The most important modification is the more complete oxidation of the sulfur compounds to sulfates, which is accomplished by gentle boiling with perchloric acid for about 15 hours. The results obtained agree with those by the Parr bomb method.

IN DETERMINING total sulfur in feeds, it is often desirable to use large samples of material containing small amounts of sulfur or to determine the sulfur content of the residue left on a filter paper. As the rapid Parr bomb method (7) cannot be used under such conditions, the use of nitric and perchloric acids for oxidizing organic matter appeared as a possible substitute for the Parr bomb sodium peroxide fusion method.

A combination of nitric and perchloric acids has been used to a considerable extent in this laboratory for the decomposition of biological materials preliminary to the quantitative determination of certain elements. Gerritz (3) used this method for digesting biological material for calcium and phosphorus determinations. Cook (2) digested feeds with nitric and perchloric acids to decompose the organic matter in preparation for the determination of manganese. St. John and Midgley (8) digested plant materials with nitric and perchloric acids as part of a rapid method for the determination of potassium in plant material.

In a preliminary study it was found that low results were obtained when sulfur was determined on a solution prepared by digesting the feed with nitric and perchloric acids as described by Gerritz (3) for the determination of calcium and phosphorus. Several modifications of this method were then investigated. Sulfur determinations were made by each method on samples of herring fish meal, soybean oil meal, and ground wheat.

#### METHODS

**METHOD A.** The nitric and perchloric acid digestion procedure developed by Gerritz (3) in this laboratory was used as starting point, and all other methods were modifications of it. It consists essentially of adding 35 ml. of concentrated nitric acid to a 2.00-gram sample of feed in a 500-ml. Kjeldahl flask, and heating gently till the material is past the colloidal stage and goes into solution. Then 10 ml. of 70% perchloric acid are added and the solution is evaporated to perchloric acid fumes and then heated till it is colorless. This method was also found satisfactory in this laboratory for manganese (2) and potassium (8).

**METHOD B.** Evidence of Kahane and Kahane (5) indicates that low results may be caused by the loss of volatile sulfur compounds before they are oxidized. Sulfur may possibly be lost as hydrogen sulfide. One gram of copper nitrate was added to the samples before digestion in an attempt to fix the sulfur unoxidized to sulfate.

**METHOD C.** This is essentially the method of Wolesensky for the determination of sulfur in rubber (9). Essential differences from Method A are that the sample is digested on a steam bath with dilute nitric acid (1 to 1) solution until the reaction subsides before the addition of concentrated nitric acid. Five mil-

Table I. Total Sulfur in Feeds

(Comparison of some nitric-perchloric acid digestion procedures and Parr bomb method)

Method	Herring Fish Meal %	Soybean Oil Meal %	Ground Wheat %
A. Method first developed in this laboratory	0.634	0.286	0.109
B. Digestion in presence of Cu-(NO <sub>3</sub> ) <sub>2</sub>	0.475	0.326	0.099
C. Start digestion with 41% HNO <sub>3</sub> , add HCl to drive off HNO <sub>3</sub> after digestion	0.545	0.302	0.114
D. Start digestion with HCl, HNO <sub>3</sub> , and H <sub>2</sub> O	0.692	0.336	0.123
E. Heat on water bath with HNO <sub>3</sub> for 24 hours	0.679	0.292	0.104
F. Boil perchloric acid gently for 15 hours	0.891	0.424	0.126
G. Combination of E and F	0.944	0.416	0.127
H. Parr bomb method	0.942	0.409	0.127



Table II. Total Sulfur in Feeds

Sample	Comparison of Parr bomb and modified nitric-perchloric acid digestion methods		G/H $\times 100$
	Parr Bomb (H) %	Nitric-Perchloric (G) %	
Feathers (0.5 gram samples, both methods)	2.093	2.034	97.2
Feather fish meal	0.958	0.950	99.2
	0.942	0.944	100.2
Crack fish meal	0.879	0.882	100.3
Whey casein	0.675	0.665	98.5
Powdered whey	0.309	0.325	105.2
Feed scraps	0.483	0.484	100.2
	0.434	0.432	99.5
Soybean oil meal	0.428	0.420	98.1
	0.409	0.416	101.7
	0.414	0.404	97.6
Alfalfa meal	0.403	0.418	103.7
Alfalfa	0.379	0.381	100.5
Alfalfa	0.132	0.132	100.0
Heat	0.127	0.127	100.0
Iron	0.128	0.125	97.7

Table III. Recovery of Sulfur of Cystine and Methionine

(By Parr bomb and nitric-perchloric acid digestion methods)			
Method	Cystine A %	Cystine B %	Methionine %
Theoretical	26.69	26.69	21.50
Parr bomb	26.58	25.61	21.27
Nitric-perchloric (Gerritz, 3)	25.65	24.46	1.55
Nitric-perchloric-modified (G)	25.76	25.24	20.65
Modified nitric-perchloric $\times 100$	96.9	98.6	97.1
Parr bomb			

ers of concentrated hydrochloric acid are added after the perchloric acid solution has become colorless and it is again heated perchloric acid fumes.

METHOD D. This is based on the micromethod of Jones (4), and consists of heating the material with 40 ml. of distilled water, 1 ml. of concentrated hydrochloric acid, and 30 ml. of concentrated nitric acid until in solution, and then proceeding as in Method A.

METHOD E. The sample is heated on the water bath with concentrated nitric acid for 24 hours, and perchloric acid is added after the material goes into solution. The nitric acid is then boiled off and the rest of the procedure of Method A followed.

METHOD F. The sample is treated with nitric and perchloric acids as in Method A. After the solution has been evaporated to perchloric acid fumes, it is boiled gently for about 15 hours. Additional perchloric acid is added when necessary to prevent evaporating to dryness.

METHOD G. This combines the modifications of Methods D and F and is essentially the method of Masters (6) with some modifications.

METHOD H. This is the Parr bomb method (7).

#### MODIFIED NITRIC AND PERCHLORIC ACID DIGESTION PROCEDURE FOR TOTAL SULFUR IN FEEDS

The data presented in Table I indicate that Method G was the only one of the nitric-perchloric acid digestion methods that gave results in good agreement with the Parr bomb method for the three samples studied. Therefore the following method was finally adopted for the determination of total sulfur in feeds by digestion with nitric and perchloric acids.

Weigh a 2.00-gram sample of the feed into a 500-ml. Kjeldahl flask, add 35 ml. of concentrated nitric acid, and heat on the steam bath till the feed goes into solution and the reaction subsides. Add 10 ml. of 70% perchloric acid and continue heating on the steam bath for a total of 24 hours. Heat the flask over a low flame till the nitric acid boils off and perchloric acid fumes are obtained. Adjust the burner so that the perchloric acid solution boils gently. Continue boiling for 15 to 16 hours, adding more perchloric acid when necessary to prevent evaporating to dryness. Usually about 5 or 10 ml. of additional perchloric acid are necessary. Cool, add 50 ml. of distilled water, and filter through a qualitative filter paper into a 600-ml. beaker. Wash the flask and filter paper well with distilled water. The volume of this stage is usually 250 to 300 ml. Add sodium hydroxide solution to the filtrate till neutral to methyl orange. Add 1 ml. of concentrated hydrochloric acid. Heat to boiling and add slowly 10 ml. of 10% barium chloride solution to precipitate the sulfate. Allow to stand for 24 to 48 hours, filter through a weighed Gooch crucible, and ignite at 800° C.

#### DISCUSSION

Some investigators have felt that the low results obtained by the previous nitric-perchloric acid digestion procedure (Method A) are caused by losses of volatile sulfur compounds through too rapid reaction or mechanical loss before they are oxidized to sulfate. A comparison of Methods A, F, and H (Table I) shows that incomplete oxidation accounts for most of the losses of sulfur. However, particularly in the fish meal, some loss was due to too rapid reaction. None of the methods employed to decrease the rapid reaction was of much value in increasing recovery of sulfur.

The total sulfur contents of a number of different types of feedstuffs were determined by both the Parr bomb and the modified nitric-perchloric acid digestion methods. A comparison of the values obtained is presented in Table II. The agreement between the two methods was very good. The nitric-perchloric acid digestion method gave an average of 99.98% as much sulfur as the Parr bomb method for the 16 samples analyzed, ranging between 97.2% for feathers and 105.2% for powdered whey. Materials high in sulfur, such as feathers, cystine, and methionine, contained 97.5% as much sulfur by the nitric-perchloric acid digestion method as by the Parr bomb method. There was a better agreement between duplicates by the nitric-perchloric acid digestion method than by the Parr bomb method.

Method A of Table I, which was the precipitation of sulfate from the nitric-perchloric acid digest of Gerritz (3), gave low results, probably because of incomplete oxidation of sulfur compounds to sulfate. It appears that while the sulfur of cystine is fairly easily oxidized by this method, that of methionine is not (Table III). To complete the oxidation it was necessary to boil for a considerable time with perchloric acid in addition to the regular digestion.

The sulfur contents of cystine and methionine were determined by the Parr bomb method (7), Method A (Gerritz, 3), and Method G (modified nitric-perchloric digestion), using 0.05-gram samples in all cases. The results are presented in Table III. Although the Parr bomb method did not give a sulfur content as high as the theoretical in any of the amino acids, it probably represents the true sulfur content. Cystine A and the methionine were commercial preparations with no statement of purity. Cystine B was prepared from hair in the laboratory and was not highly purified. The modified nitric-perchloric acid method gave 96.9 to 98.6% as much sulfur on these compounds as the Parr bomb method. The previous nitric-perchloric acid method of digestion gave a good recovery from cystine but very little from methionine. This agrees with the findings of Callan and Toennies (1) for the alkaline permanganate method.

The total time required for making a determination by the nitric-perchloric acid digestion method on a set of six samples in duplicate was 6 days from the time the samples were weighed out till the results were obtained. This contrasts with 4 days by the Parr bomb method. However, the nitric-perchloric acid digestion method required about 5 hours less of the analyst's actual working time than the Parr bomb method.

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# Determination of Vitamin A in Dehydrated Eggs

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A spectrochemical method for the determination of vitamin A in dehydrated eggs is described. The procedure involves careful control of analytical conditions, followed by the use of a correction factor required because of the absorption of ultraviolet radiation by the carotenoid pigments present. The correction must include the effects of isomerization of the pigments caused by the analytical process, and has been determined on the basis of the two principal pigments present, luteol and zeaxanthol. The absorption spectra of these two pigments, in absolute ether, and the specific absorption coefficients at their wave lengths of maximum and minimum absorption are presented.

THE increased emphasis on the nutritional value of foods and food products has increased, among other things, the need for adequate methods of analysis for vitamin A. Biological methods are both time-consuming and expensive. Chemical methods seem to be limited at present to two procedures: the well-known Carr-Price (2) antimony trichloride reaction, or analysis based on the absorption of ultraviolet radiation by the vitamin A. In the case of dehydrated eggs, a product that is being studied in this laboratory, the pigments present influence the results, and adequate correction factors are required. This paper presents a method of analysis based on the ultraviolet absorption of vitamin A, with a suitable correction for the presence of the two principal carotenol pigments. Duplicate samples agree within 10%, and a few samples on which bioassays were available give reasonable checks. Extraction procedures are similar to those used for the extraction of vitamin A from other products.

## PROCEDURE

**EXTRACTION OF VITAMIN A.** A 10-gram sample of dehydrated egg is placed in a Waring Blendor and extracted for 10 minutes with 100 ml. of peroxide-free ether. The sample is filtered on a Büchner funnel and washed with several small portions of ether. The egg powder is then replaced in the Blendor and re-extracted with 60 ml. more of ether for 2 minutes. A third extraction for another 2 minutes is usually required. This extraction procedure removes all the vitamin A and practically all the yellow pigments.

**PREPARATION OF EXTRACT FOR ANALYSIS.** The combined ether extracts are placed in a 500-ml. round-bottomed flask, to which a few glass beads have been added to prevent bumping, and evaporated on a steam cone, under the reduced pressure produced by a water pump, to a final volume of 15 ml. (It is important that this evaporation be carried to the same volume each time.) Twenty milliliters of 95% methanol and 5 ml. of a saturated aqueous solution of potassium hydroxide are added to the residue in the flask, and the mixture is heated under reflux for 10 minutes. The sample is cooled immediately; 40 ml. of water are added and the contents are then transferred to a 500-ml. separatory funnel. The flask is rinsed with an additional 40 ml. of water, followed by rinsings with two 25-ml. portions of ether, which are also added to the contents of the funnel. The methanol-water solution of vitamin A is then extracted with 25-ml. portions of ether until the ether layer is colorless. It has been shown that the absence of yellow color in the ether phase is an indication of complete extraction of vitamin A.

The ether extract is washed with water until the wash is neutral to litmus, and then dried over anhydrous sodium sulfate. The extract is filtered through sodium sulfate into a volumetric flask. A crystal-clear, yellow filtrate should result.

**SPECTROPHOTOMETRIC ESTIMATION OF VITAMIN A.** The sample is made up to volume (250 ml.), and its optical density is determined on a Beckman (3) spectrophotometer at 326 m $\mu$  for vitamin A and at 450 m $\mu$  for total yellow color. Fifteen per cent of the density at 450 m $\mu$  is subtracted from the density at 326 m $\mu$  as a correction for absorption at 326 m $\mu$  due to the yellow pig-

ments present. The vitamin A present may be calculated by means of the following equation:

$$\text{Micrograms of vitamin A per gram} = \frac{D_{326} - 15\% D_{450}}{0.176} \times \frac{\text{volume in ml}}{\text{weight in gram}}$$

## DISCUSSION

The extraction of the ether-soluble fraction from dehydrated eggs was somewhat more difficult than from many other food products. Several methods of extraction were tried before the method described was finally chosen. The Waring Blendor treatment apparently removed all the vitamin A. Further extraction produced no increase in the quantity of vitamin A removed.

Evaporation of the ether extract must be carefully controlled and brought to the same final volume in each case, since carotenoid-type pigments isomerize under the influence of heat (15). The isomerization of the pigments produces changes in their spectral absorption curves, which affect the absorption in the ultraviolet region where correction for their presence is necessary. The time of saponification is held constant for the same reason.

Studies on the pigments of eggs (6, 7, 8) show that, although pigmentation may be controlled by feeding, the pigments in eggs produced by hens on a normal diet contain over 90% luteol and zeaxanthol. The remaining pigments are primarily cryptoxanthol and carotene.

Several different samples of dehydrated eggs were extracted with ether and the pigments adsorbed on a column of 1 part magnesia (Micron brand No. 2641) and 2 parts Hyflo Super-Cel. The column was developed with a mixture of 12% acetone in Skellysolve B. Five bands were produced, four of which were identified as the pigments named above; the fifth, which was held tightly at the top of the column, was undoubtedly a mixture of very small amounts of oxidized material. The four identified pigments were quantitatively determined by removing them from the column, cutting out the various bands, and eluting the pigments with acetone. Skellysolve B was then added and the acetone removed by washing with water. The extract was dried over anhydrous sodium sulfate and made up to a convenient volume. The amounts of pigments present were then determined, using the specific absorption coefficients and wave lengths of maximum absorption as given by Zscheile *et al.* (18). Slight errors may be expected in the cases of luteol and zeaxanthol, since the data of Zscheile were obtained in an ethanol solution. No coefficients were available for these two pigments in petroleum ether or hexane. A comparison of the total pigment present as compared to the sum of the fractions eluted from the column indicated some loss (10 to 15%), which is assumed to be distributed proportionally through the components.

In all cases the sum of the concentrations of luteol and zeaxanthol accounted for more than 90% of the total pigments, luteol being present in the larger amounts. The amount of luteol in different samples varied from 63 to 76% of the total pigments, while the zeaxanthol varied from 32 to 20%. The cryptoxanthol amounted to 3 to 5% and the carotene to 2 to 4% of the total. The remainder was in the band at the top of the column.

Since the luteol and zeaxanthol accounted for approximately 90% of the total pigment, corrections for their presence were obtained. The other pigments were not included in the establishment of the correction factor because of the small amounts present. It has been shown, however, that  $\beta$ -carotene affects analytical data in the same general way (10). In order to obtain corrections, luteol was crystallized from alfalfa meal and zeaxanthol from dehydrated eggs, the crystallizations and separations being



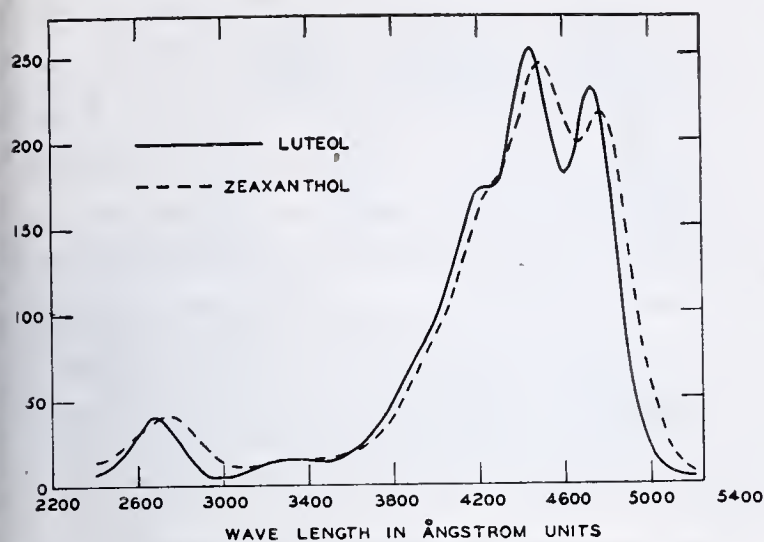


Figure 1. Absorption Spectra of Luteol and Zeaxanthol in Absolute Ether

Table I. Absorption Values of Luteol and Zeaxanthol in Ether

	Luteol		Zeaxanthol	
	$\lambda$ (m $\mu$ )	$\alpha$	$\lambda$ (m $\mu$ )	$\alpha$
Maxima	473.5	230	476.0	217
	444.5	254	450.0	246
	422.0	173	...	...
Minima	460.0	181	468.0	199
	425.0	172	...	...

Table II. Effect of Analytical Procedure on Ultraviolet Absorption of Luteol and Zeaxanthol

Pigment	Treatment	$D_{456 \text{ m}\mu}$	$D_{326 \text{ m}\mu}$	$\frac{D_{326 \text{ m}\mu}}{D_{456 \text{ m}\mu}} \times 100$
Luteol	None	0.675	0.042	6.2
	Through analytical procedure	0.778	0.122	15.7
		1.500	0.191	12.7
		0.948	0.150	15.8
Zeaxanthol	None	0.715	0.045	6.3
	Through analytical procedure	0.165	0.028	16.9
		0.118	0.020	16.9
1/3 luteol plus 1/3 zeaxanthol	Through analytical procedure	0.770	0.116	15.1
		0.635	0.095	15.0

carried out as described by Zscheile *et al.* (18). After recrystallization their absorption spectra were determined, in ether, from 510 to 240 m $\mu$ . These absorption curves are similar to those of White, Brunson, and Zscheile (11) over the range used, although they should not be compared too closely because different solvents were used. The wave lengths of the maxima and minima together with the corresponding specific absorption coefficients, in diethyl ether, are presented in Table I.

It will be seen in Figure 1 that the curves intersect near 450 m $\mu$  and are close together at 326 m $\mu$ , the wave length at which the vitamin A maximum occurs. The absorption at 326 m $\mu$  is about 6.2% of the absorption at 450 m $\mu$ . This is about the same as the ratio reported by Deuel *et al.* (4), which they obtained on a sample of luteol furnished by Zechmeister and used to establish a correction for egg pigments. They used a mixed solvent of low-boiling petroleum ether and isopropanol, while the authors' data were obtained with ether as the solvent.

However, 6.2% should not be used as the correction for these pigments, since isomerization raises the absorption peak in the ultraviolet. Zechmeister (12, 14) has recently presented data on luteol and zeaxanthol showing this to be true of these two pigments as well as others. To determine the extent of this effect, a sample of pure luteol was carried through the analytical procedure and the ratio of the absorption readings at 450 and 326 m $\mu$  determined. The same procedure was followed with zeaxanthol and a mixture of the two pigments in the approximate proportions in which they occur in eggs. The results, tabulated in

Table II, show that the true correction is about 15% of the absorption at 450 m $\mu$ . This correction is valid only if the heating times and procedures used are those given here. The 15% correction obtained for these pigments is very similar to a correction applied to butterfat by Baumann *et al.* (1) several years ago. Longer periods of heating cause additional isomerization and consequently would affect the reading.

The absorption coefficient of 0.176 in the equation used for the calculation of the vitamin A concentration has been determined on a sample of crystalline vitamin A generously furnished by Distillation Products, Inc. This figure is close to the average obtained by Zscheile and Henry (15), although Zscheile *et al.* (17) later report another sample as having a value of 0.1825, with an absorption peak at 324 m $\mu$ . The absorption maximum obtained with this sample of vitamin A occurred at 326 m $\mu$  when the vitamin A was dissolved in ether.

The recovery of vitamin A was fair. Recoveries of added vitamin A averaged slightly over 90%. An ether solution of vitamin A alcohol carried through the procedure alone gave slightly higher recovery percentages.

The usual precautions regarding light were taken, although amber glassware, as recommended by Embree (5) was not available. All steps in the procedure were carried out in a darkened room. Sunlight was excluded entirely and diffuse light used for illumination. Whenever possible, the samples were placed in a cupboard, in the dark. Spectrophotometric readings were taken the day of extraction, or kept in refrigeration until read. The usual checks on solvents also were made. Ether was free of peroxides and the alcohol free of aldehydes. Glassware was thoroughly cleaned between analyses. Special care is needed to remove all fatty materials which might become rancid and destroy vitamin A.

A bioassay comparison with the proposed spectrophotometric procedure on the three samples which were available showed fair agreement (see Table III). Similar agreement was obtained by Zscheile *et al.* (16) on samples of butterfat analyzed by spectrophotometric methods, using a somewhat similar arbitrary correction factor. The comparison with the bioassay is made assuming that the vitamin A has a potency of 4,300,000 U.S.P. units of vitamin A per gram. No determination of the additional vitamin A effects of other substances present is included.

Table IV presents typical data obtained on several samples analyzed by the proposed method. Although the correction required because of the presence of the yellow pigments is large, the

Table III. Comparison of Bioassay and Spectrophotometric Analysis

Sample	Bioassay I.U./g.	Spectrophotometric Analysis I.U./g.
1	36.3	35.1 33.1 35.4
2	31.6	35.5 49.5 46.8
3	58.5	

$$\text{I.U./g.} = 4.3 \times \gamma/\text{g.}$$

Table IV. Typical Spectrophotometric Data Obtained by Described Procedure

Sample	$D_{450 \text{ m}\mu}$	$D_{326 \text{ m}\mu}$	Correction for Carotenols (15% $D_{450 \text{ m}\mu}$ )	Corrected $D_{326 \text{ m}\mu}$	Micrograms of Vitamin A per Gram
1	0.640	0.183	0.096	0.087	12.3
	0.614	0.184	0.093	0.091	12.9
2	0.560	0.157	0.084	0.073	10.4
	0.553	0.158	0.082	0.076	10.8
3	0.421	0.121	0.063	0.058	8.2
	0.405	0.116	0.061	0.055	7.8
4	0.155	0.081	0.023	0.058	8.2
	0.160	0.092	0.024	0.068	9.6
5	0.143	0.097	0.021	0.076	10.8
	0.145	0.089	0.021	0.068	9.6



method gives reasonable, reproducible results. In a series of 120 samples under various treatments and storage conditions, values ranging from 13.6 to 6.3 micrograms per gram were obtained, well within the range of values obtained by other methods of analysis.

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## Determination of Carbon

### Simplification of Low-Pressure Combustion Apparatus

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The interference of water vapor in the apparatus for the low-pressure combustion method of determining carbon in iron and steel has been investigated and changes in operating technique have minimized this interference. The equipment has been simplified further, resulting in a greater speed of manipulation. In over 600 determinations made with the new equipment the results have shown good agreement with those obtained on earlier forms of apparatus.

THE composition of the blank gases obtained in the low-pressure combustion equipment described by Murray and Ashley (2) has been determined by means of vapor pressure curves. A considerable fraction of this gas was found to be water vapor which moved from the dry ice trap to the liquid nitrogen trap during the pumping period.

The authors have replaced the complex mercury cut-off system in the previous apparatus (2) with a single stopcock. Two carbon dioxide measuring systems are used with one combustion vessel. These changes have decreased the time required for a determination, so that 10 to 15 samples can be run in an 8-hour shift on one unit of this type.

#### ANALYSIS OF BLANK GASES

A carbon analysis unit as described by Murray and Ashley (2) was equipped with a single trap at  $T_5$ . Vapor pressure curve analysis of gases frozen out in this trap was made by a procedure similar to that described by Sebastian and Howard (3).

The vaporization curve of mixtures of carbon dioxide, sulfur dioxide, and water was measured in the equipment in order to prove that these gases could be identified. This mixture was introduced into the system by warming sodium bicarbonate and sodium bisulfite which were placed in a branch of a loading arm prior to evacuation of the system. Curves obtained for two different mixtures of these gases are shown in Figure 1.

A new platinum crucible with beryllia lining crucible was used for the investigation of the origin and composition of the blank. A vapor pressure curve analysis was carried out on each blank on this crucible assembly. These blanks, run according to the operating procedure of Murray and Ashley (2), were found to contain carbon dioxide and water vapor, as shown in Table I. No evidence of the presence of other gases was found. From these and similar data it appears that water vapor is responsible for from  $1/3$  to  $1/2$  of the blank when it is in the low range used for an actual carbon analysis (blank  $<0.001\%$  carbon on a 0.5-gram sample).

During collection of these blank gases, the combustion vessel and dry ice trap,  $T_4$ , were connected during the entire pumping period until the pressure had been reduced to  $10^{-5}$  mm. of mercury. Gurry and Trigg (1) avoid the transfer of water vapor from the dry ice trap to the liquid nitrogen trap by closing off the former when the pressure has been reduced to 0.1 mm. of mercury and then exhausting the actual measuring system to  $10^{-5}$  mm.

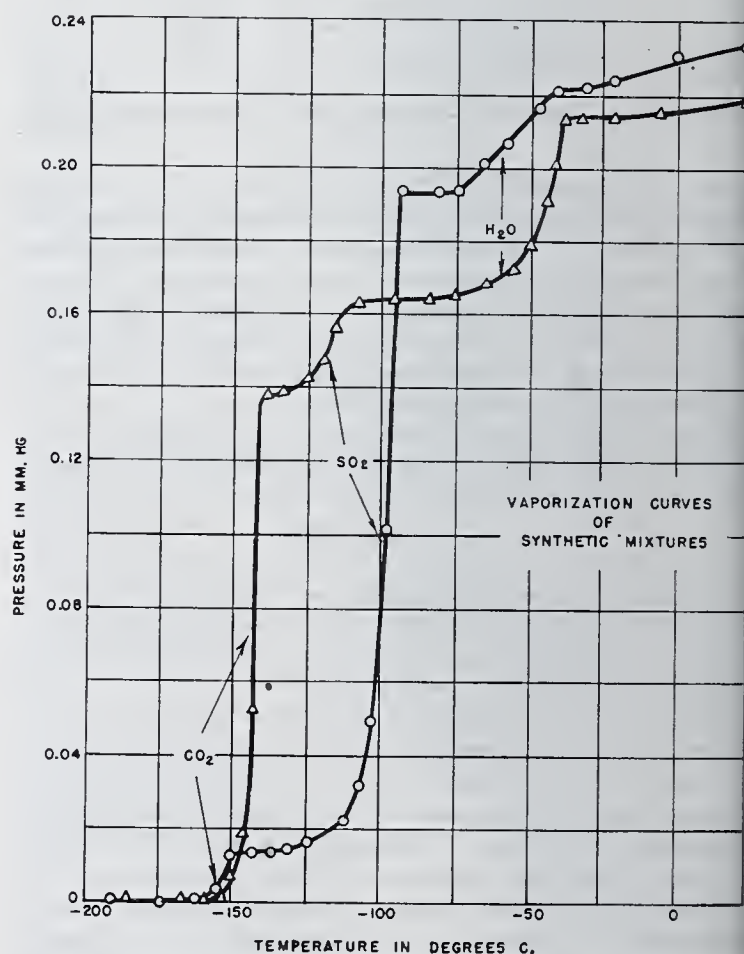


Figure 1



Table I. Composition of Blank Gases

No. of Blank Run	Pressure		Calcd. as Wt. % Carbon on 0.5 Gram Sample	
	CO <sub>2</sub> Mm.	H <sub>2</sub> O <sup>a</sup> Mm.	CO <sub>2</sub> %	H <sub>2</sub> O <sup>a</sup> %
1	0.598	0.097	0.021	0.003
2	0.110	0.047	0.0039	0.0016
3	0.0735	0.025	0.0026	0.00088
4	0.0100	0.0048	0.00035	0.00017

Not accurate because of behavior of H<sub>2</sub>O vapor in gage. Pressure read same mark of gage as CO<sub>2</sub> pressure.

In the present investigation of the Murray and Ashley (2) apparatus, tube *J* was changed and sealed into the multiple cut at *L*<sub>2</sub>. This allowed the system of Gurry and Trigg (1) to be followed by pumping after combustion, with the mercury below until the pressure was 0.1 mm. of mercury and then raising the mercury above *L*<sub>2</sub> and exhausting the measuring system to 10<sup>-5</sup> mm. of mercury.

In Figure 2 the effect of the water vapor transfer from trap to trap *T*<sub>5</sub> is illustrated by vapor pressure curves.

Curve *A* was obtained from a blank in which the combustion vessel and *T*<sub>4</sub> were pumped to 10<sup>-5</sup> mm. of mercury (20 minutes) and then the measuring system. This curve shows that after water vapor does transfer from *T*<sub>4</sub> to *T*<sub>5</sub> under these conditions of pumping.

Curve *B* represents another blank in which pumping of *T*<sub>4</sub> was stopped when the pressure reached 0.1 mm. of mercury and only the measuring system was evacuated to 10<sup>-5</sup> mm. This curve shows that this method of operation eliminates the transfer of an appreciable quantity of water vapor into *T*<sub>5</sub> from *T*<sub>4</sub>.

The series of *C* curves was obtained, after exhausting the gas from *B*, by pumping the entire system for 30 minutes with *T*<sub>4</sub> and cooling to their indicated temperatures. Thus these curves represent the transfer of water vapor from *T*<sub>4</sub> to *T*<sub>5</sub> during a 30-minute pumping period. *C*<sub>1</sub> was read on the high-compression mark of the McLeod gage, *C*<sub>4</sub> on the low-compression mark, and *C*<sub>3</sub> on intermediate marks.

#### DESIGN OF NEW APPARATUS

Since the apparatus described by Murray and Ashley (2) contained two stopcocks per unit, it seemed worth while to attempt the introduction of one more stopcock and thus eliminate the complex mercury cut-off system entirely. Shepherd (4) pointed out that no difficulty had been encountered from stopcocks in the previous apparatus (2) and trap *T*<sub>4</sub> should remove any contaminating material (derived from the grease) from the oxygen entering the combustion vessel.

The new apparatus is shown diagrammatically in Figure 3. The oxygen purification system is similar to that used previously (1). Only the *A* measuring system is shown in the diagram, but the *B* system is identical in construction. Stopcocks *S*<sub>3A</sub> and *S*<sub>4A</sub> replace the mercury cut-offs in two units of the earlier apparatus. Bulb *X*<sub>A</sub> is of such a volume that the total calibrated volume (*S*<sub>3A</sub> - *S*<sub>4A</sub>) is about 500 ml. thus making it possible to handle samples containing 0.001 to 0.10% carbon in this single intermediate volume rather than the two volumes used previously. The two measuring systems, *A* and *B*, and one combustion vessel constitute a unit. In the authors' laboratory two units of this type are built on one 2 × 6 foot table with the oxygen purification system serving both units, as indicated by the dotted tube above *S*<sub>2</sub>.

All stopcocks are hollow-plug precision-ground obtained from W. K. and Krebs, New York, N. Y. *S*<sub>1</sub>, *S*<sub>2</sub>, *S*<sub>3</sub>, and *S*<sub>4</sub> are 10- to 12-in. bore. This large size is used because there is less danger of sticking and because the large bore does not plug easily with grease and stop the pumping of gases at low pressures. *S*<sub>5</sub> and *S*<sub>6</sub> are of smaller bore (4 to 6 mm.). All stopcocks are greased with Apiezon L.

Calibration of the known volume is made in the manner described by Murray and Ashley (2).

#### OPERATION OF EQUIPMENT

Reference is made to measuring system *B*, although it is not shown in Figure 3.)

With *S*<sub>1</sub>, *S*<sub>5A</sub>, and *S*<sub>5B</sub> closed, evacuate the entire system to 10<sup>-5</sup> mm. of mercury pressure. Cool *T*<sub>1</sub>, *T*<sub>2</sub>, *T*<sub>5A</sub>, and *T*<sub>5B</sub> with liquid nitrogen and *T*<sub>3</sub> with dry ice-acetone mixture. Close

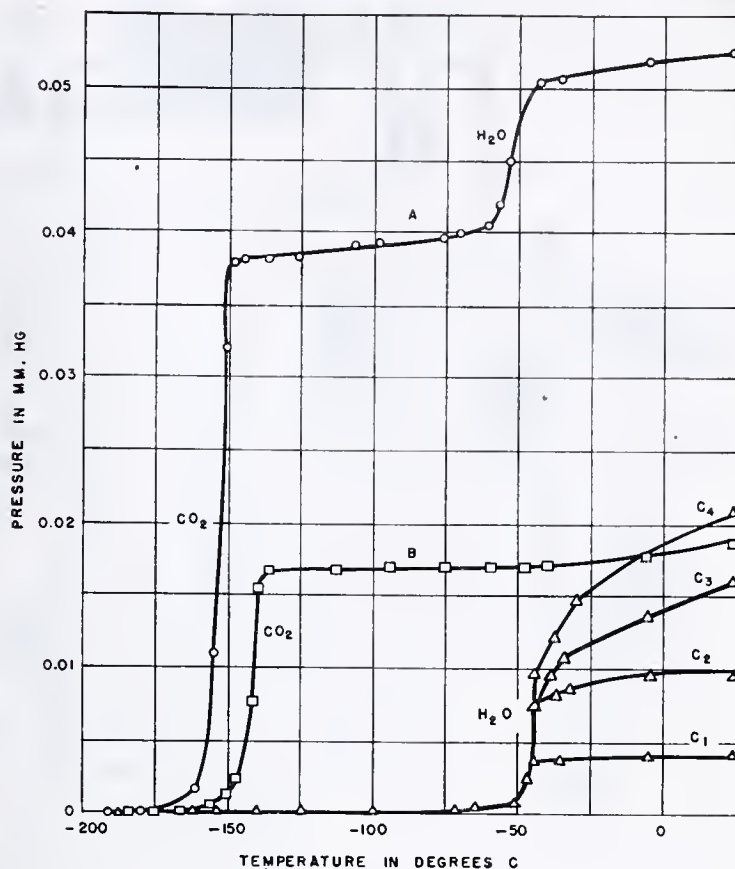


Figure 2.

*S*<sub>3B</sub> and *S*<sub>4A</sub>, then admit oxygen slowly through *S*<sub>1</sub> until the pressure in the combustion vessel and measuring system *A* is 15 to 20 cm., as indicated by depression of the mercury column below gage *M*<sub>A</sub>. Close *S*<sub>2</sub> and cool *T*<sub>4A</sub> with liquid nitrogen. Burn the sample in the manner described previously (2), then open *S*<sub>4A</sub> very slightly and pump the gas slowly from the combustion vessel through *T*<sub>4A</sub>. The rate of removal of gas will be indicated by the rate of mercury rising from the reservoir below *M*<sub>A</sub>, and must be slow, so that all the carbon dioxide is frozen out in *T*<sub>4A</sub>. When the mercury ceases to rise below *M*<sub>A</sub>, open *S*<sub>4A</sub> completely and pump until the pressure is 0.1 mm. (The time required for this initial pumping down to 0.1-mm. pressure is usually about 5 minutes.) Close *S*<sub>3A</sub> and evacuate measuring system *A* to 10<sup>-5</sup> mm. of mercury pressure (time about 5 minutes). Close *S*<sub>4A</sub> and expand the carbon dioxide into the known volume *S*<sub>3A</sub> - *S*<sub>4A</sub>. Measure the pressure of this gas at room temperature, then open *S*<sub>4A</sub> and exhaust measuring system *A*.

As soon as *S*<sub>3A</sub> is closed after burning the first sample, open *S*<sub>3B</sub> and close *S*<sub>4B</sub>. Admit oxygen to the combustion vessel and measuring system *B* and burn another sample while system *A* is being pumped and the gas measured. By the time system *A*

Table II. 8-Hour Record of Analyses Showing Blanks

(New crucible used, all blanks recorded)

No. of Burning	Measuring System Used	Sample	% Carbon
1	A	Blank	0.0246
2	B	Blank	0.0006
3	A	Blank	0.0004
4	B	Blank	0.0003
5	A	Fe-Ni-Co alloy <sup>a</sup>	0.0205
6	B	Fe-Ni-Co alloy	0.0093
7	A	Fe-Ni-Co alloy	0.0079
8	B	Fe-Ni-Co alloy	0.0274
9	A	Blank	0.0003
10	B	Si steel <sup>b</sup>	0.0044
11	A	Blank	0.0003
12	B	Si steel	0.0041
13	A	Si steel	0.0042
14	B	Si steel	0.0056
15	A	Blank	0.0004
16	B	Blank	0.0005

<sup>a</sup> Four samples of Fe-Ni-Co were different.

<sup>b</sup> Four samples of Si steel were same material.



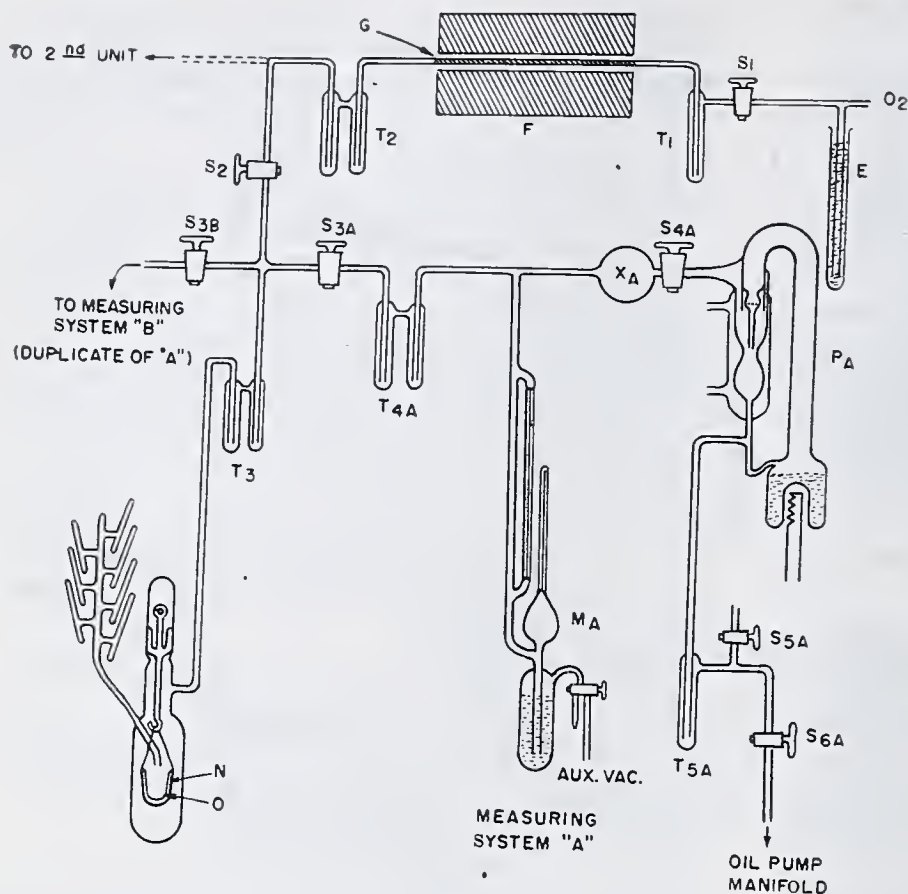


Figure 3. Apparatus

is ready for the third sample, system *B* will be pumping with  $S_{3B}$  closed, so that the combustion vessel will be ready for *A*.

Using this scheme of operation, about 0.1% of the gas from the first sample remains in the combustion vessel when oxygen is added for burning the second sample. If the first sample contained 0.1% carbon, the carbon dioxide remaining in the combustion vessel would correspond to 0.0001% carbon, which is negligible as a loss from the first sample or as an addition to the second sample. Even after running 10 samples in this manner the accumulation of carbon dioxide in the combustion vessel would be equivalent to only 0.0001001% carbon. Since the samples usually contain less than 0.02% carbon, this error is entirely negligible.

Interference by water transferring from  $T_3$  to  $T_{4A}$  or  $T_{4B}$  has not been encountered since this method of pumping has been employed.

#### ROUTINE OF OPERATION

One unit with two measuring systems can be handled by one operator very easily. It is possible to determine carbon on 10 to 15 samples in 5 to 6 hours in this manner.

The routine established has been that of preparing a loading arm containing 10 to 15 samples and sealing this onto the combustion vessel late in the afternoon. The apparatus is pumped out and trap  $T_3$  flamed with a torch for a few minutes while pumping. The next morning blanks are run (2 or 3 as required) and then all the samples analyzed. By mid-afternoon the loading arm is empty and the apparatus is shut down and air admitted. Another loading arm filled with samples is sealed on, the crucible changed if necessary, and the apparatus exhausted and  $T_3$  flamed. By following this routine it has been possible to turn out 10 to 15 analyses every day without difficulty.

Flaming of trap  $T_3$  after each time that air is admitted to the apparatus (or once daily) is important to prevent water vapor and sulfur compounds from accumulating in this trap.

#### BLANK

The blank on this new equipment is easily reduced to a value equivalent to 0.0005% carbon or less on a 0.5-gram sample. The

data given in Table II are representative of normal operation of a unit, although more blanks and fewer samples were run in this case than usual in order to illustrate the maintenance of a low blank. These data were accumulated in a regular 8-hour shift.

#### RESULTS WITH REFERENCE SAMPLES

The data given in Table III represent all results obtained on two reference samples during the first six weeks of operation of the new apparatus. One of the reference samples is Bureau of Standards Sample 55a, a very pure open-hearth iron, and the other is a 3% silicon steel sample which has been used by the authors for reference.

The average value of 0.0108% carbon found on B. of S. 55a is to be compared with the value 0.0121% carbon previously reported from this laboratory (2) and the values 0.0108% carbon reported by Wooten and Guldner (5) and 0.0108% reported by the U. S. Steel Corporation (J. Austin) (2). It is evident that this lower value obtained by the new method is in better agreement with the values obtained by other users of the low-pressure combustion method than the value obtained at Pittsfield on the previous apparatus of Murray and Ashley (2).

The one very high value (0.0086% carbon) found on the silicon steel sample probably resulted from contamination and was rejected in calculating the average and the average deviation.

These data of Table III indicate that the new simplified apparatus yields satisfactory results. The values reported have not been corrected for the blank because it is difficult to establish a fixed blank to be used for such a correction. A blank less than 0.001% carbon and a precision of  $\pm 0.001\%$  carbon has been considered satisfactory for the routine analysis of this laboratory and the new apparatus meets these requirements very satisfactorily.

Table III. Carbon in Reference Samples

(Not corrected for blank)			
B. of S. Sample 55a %	Silicon Steel %	B. of S. Sample 55a %	Silicon Steel %
0.0106	0.0035	0.0108	0.0032
0.0106	0.0037	0.0103	0.0045
0.0116	0.0038	0.0113	0.0044
0.0104	0.0034	0.0113	0.0042
0.0104	0.0034	0.0107	0.0044
0.0110	0.0042	0.0121	0.0041
0.0111	0.0045	0.0109	0.0056
0.0107	0.0032	0.0106	0.0043
0.0105	0.0049	0.0103	0.0043
0.0103	0.0039	0.0098	0.0036
0.0103	0.0051	0.0100	0.0044
0.0111	0.0032	0.0103	0.0028
0.0109	0.0039		0.0052
0.0114	0.0033		0.0038
0.0104	0.0031		0.0036
0.0119	0.0086 <sup>a</sup>		0.0036
0.0104	0.0037		0.0029
0.0107	0.0032	Av. 0.0108	Av. 0.0039
		Av. deviation 0.00043	Av. deviation 0.00055

<sup>a</sup> Value rejected, not used in calculating average or average deviation.

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# Qualitative Study of the Color Reaction of Phosphomolybdic Acid

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THIS short paper reports the color reaction of phosphomolybdic acid with six sugars, twelve metals, and thirteen other reducing agents.

REAGENT. Phosphomolybdic acid solution (test solution), 1 g. of  $P_2O_5 \cdot 24 MoO_3 \cdot xH_2O$  (Schering-Kahlbaum), dissolved in 100 ml. of water.

Table I. Color Reaction of Phosphomolybdic Acid and Molybdate with Sugars

Sugar	Test Solution (3 Ml.)	Test Solution (3 Ml.) + 3 N $H_2SO_4$ (1 Ml.) <sup>a</sup>	1% Ammonium Molybdate (3 Ml.) <sup>b</sup>	1% Ammonium Molybdate (3 Ml.) + 3 N $H_2SO_4$ (1 Ml.) <sup>b</sup>
Sucrose	Light green	Light green	None	Blue
Maltose	Light green	Green	None	Blue
Lactose	Bluish green	Deep bluish green	None	Deep blue
Glucose	Green	Green	Blue	Bluish green
Fructose	None	Light green	None	Blue
Sucrose <sup>c</sup>	Green	Deep bluish green	None	Deep blue

<sup>a</sup> Reagent retained yellow color after boiling 3 minutes.

<sup>b</sup> Reagent remained colorless after boiling 3 minutes.

<sup>c</sup> Sucrose solution gave no precipitate of cuprous oxide when boiled with Fehling's solution.

Table II. Color Reaction of Phosphomolybdic Acid with Metals and Reducing Compounds

Reducing Substance	Color	Remarks
Iron (turnings)	Green, bluish then blue	Color changed very slowly
Iron (powder)	None	Blue color produced when concd. $H_2SO_4$ added and mixture warmed
Copper (dust)	Blue	Color produced immediately
Copper (powder)	Green, bluish then blue	Color changed very slowly
Copper (granules)	Green, bluish then blue	Color changed very slowly
Copper (granules)	Green, bluish then blue	
Copper (granules)	Blue	Color developed slowly
Copper (granules)	Bluish green	Color developed slowly
Copper (granules)	None	Bluish green color produced when dilute $H_2SO_4$ added and mixture boiled
Copper (granules)	Bluish green	Color developed slowly
Copper (granules)	Blue	Color developed slowly
Sodium sulfate (0.1%)	Bluish green	
Sodium ammonium sulfate	Bluish green	
Stannous chloride (1% in 0.1% HCl)	Blue	
Sodium bisulfite	Green, bluish then blue	Color changed very slowly; more rapidly if solution was boiled
Sodium thiosulfate	Green, bluish then blue	Color changed slowly; more rapidly if solution was warmed
Sodium hydrosulfite	Deep blue	
Potassium iodide	None	Bluish green color developed when solution was slightly warmed
Potassium ferrocyanide	Bluish green	Color changed to reddish brown when $H_2SO_4$ added
Nitroxylamine hydrochloride	None	Bluish green color developed when solution was boiled
Hydrazine hydrochloride	Green, bluish then blue	
Hydrazine hydrochloride	Deep blue	
Hydroquinone	Blue	
Resorcinol	Blue	

PROCEDURE. Color Reaction of Phosphomolybdic Acid and Molybdate with Sugars. One milliliter of the sugar solution (5%) was added to the proper amount of the reagent. The whole was boiled for 3 minutes, the volume of the solution being kept unchanged by constantly adding water to it. Six common sugars were tested against phosphomolybdic acid, acidulated phosphomolybdic acid, ammonium molybdate, and acidulated ammonium molybdate. The results are tabulated in Table I.

Color Reaction of Phosphomolybdic Acid with Metals and Reducing Compounds. The metal or the solution of the reducing compounds (1 to 2 ml.) was added to 3 ml. of the test solution. The color reaction usually took place at room temperature; in only a few cases was warming or boiling necessary. The reducing compounds were 1% aqueous solutions, if not otherwise specified. The results are tabulated in Table II.

The phosphomolybdic acid did not give color reaction with formaldehyde, formic, lactic, and oxalic acids even when the solution was boiled.

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## Separation of Catalysts from Hydrogenation Reaction Mixtures

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DURING the course of a research investigation, the problem of the removal of large quantities of hydrogenation catalysts, such as Raney nickel, platinum, or palladium, from the reduction medium was encountered. The usual methods involve filtration through paper by suction or use of the centrifuge. The former operation is by no means satisfactory, since the finely divided catalyst often passes into the filtrate, and, in addition, the pyrophoric nature of these metals produces sparking and charring of the filter paper. This becomes a definite fire hazard when large quantities of catalyst and inflammable solvent are handled. Removal of the catalyst by centrifugation often gives better results, but involves more manipulation, which may be deleterious to easily decomposed reduction products.

A procedure which has given good results but does not suffer from the deficiencies listed above, entails the use of a filter aid, such as Dicalite 4200, or its equivalent, spread in a layer about 1 cm. thick over filter paper seated in a Büchner funnel of appropriate size. This technique, which is by no means a new one in the industrial or analytical field, gives sparkling filtrates completely free of catalyst, and minimizes the possibility of ignition of the solvent or metal.

Test runs on various compounds have indicated that little if any material is adsorbed during this treatment, and the noble metal catalysts may be recovered without difficulty.

This procedure is advantageously modified in most cases by including the filter aid with the compound to be hydrogenated so that it is present during hydrogenation. This tends to give a better suspension of the catalyst and in many cases a better color of the finished product is obtained.



# Instrument for Measuring Changes in Texture of Dehydrated Fish

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A NUMBER of mechanical devices for the measurement of tenderness of various kinds of food products such as peas and beefsteak have been described but none seems exactly suitable for dehydrated fish. The undesirable feature, inherent in an organoleptic method, is that the tester cannot accurately carry over from one testing period to another the standards for the degrees of texture evaluation. Thus any observations regarding the course of change occurring during an extended period of storage might be subject to considerable error. In order to eliminate the irregularities to which organoleptic tests are so susceptible and to be more directly applicable to the need, an instrument has been devised to record numerical values proportional to the changes in texture occurring in dehydrated fish during storage.

The new instrument consists essentially of a set of shearing plates or jaws, a supporting stand, a spring scale of 54.5-kg. (120-pounds) capacity, and a geared-down winch (Figure 1). The shearing jaws consist of 5 upper and 6 lower tool steel plates with square ground edges, 0.47 cm. ( $\frac{3}{16}$  inch) thick by 3.75 cm. (1.5 inches) wide, by 12.5 cm. (5 inches) long for the upper and 10.94 cm. (4.375 inches) for the lower. These are so arranged that the upper plates nest between the lower ones with approximately 0.025-mm. (0.001-inch) clearance, so that a positive shearing action is effected. As may be seen in Figure 2 the sample compartment, which is 5.16 cm. ( $\frac{21}{16}$  inches) long by 2.5 cm. (1 inch) in diameter, is formed by cutting away a portion of the plates of the lower jaw and providing a shield on each side. The cut-away portion of the top jaw exactly coincides with that of the lower jaw when they are closed. Thus the sample compartment can be easily cleaned by raising the shields at the sides and brushing out the sample residue.

The sample to be tested is placed loosely and evenly in the compartment with the jaws open and the shields lowered into position. The upper jaw is then lowered until it rests on the sample, the spring scale is hooked to it, and a pulling force is applied to the scale by means of a cable fastened to the winch below. The force necessary to shear the sample is read directly in pounds from the dial. As the sample is sheared the upper jaw drops suddenly, thus releasing the pressure on the scale, and the maximum reading is registered by means of a friction hand. The scale is then unhooked, the shields are raised, and the sample residue is brushed out of the compartment into the waste can

located directly below the jaws. The upper jaw is again raised, the shields are lowered into position, and the instrument is ready for another sample.

Preliminary tests indicated that to get uniform data the sample must be of uniform size and a uniform rehydration procedure must be followed in its preparation.

Ten grams of the dehydrated fish are allowed to rehydrate in 60 ml. of water at room temperature for 30 minutes. Temperature of rehydration as shown by Hamm, Butler, and Heerdts (1) is not critical, so that exact control of temperature during rehydration is not necessary. The reconstituted sample is then drained free of water on an inclined screen for 2 minutes and 10-gram samples of the reconstituted material are used for the measurements.

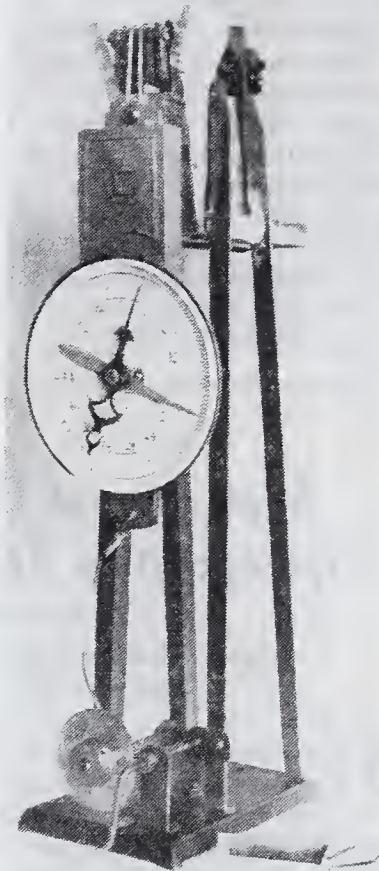


Figure 1. General View of Instrument

Table I. Change in Texture of Dehydrated Fish upon Storage Indicated by Instrument Readings

Original	After 14 Days	After 30 Days
14.0	23.6	32.8
14.5	24.6	38.8
16.0	28.0	36.2
16.0	24.0	35.4
13.5	22.8	38.2
17.5	25.8	35.4
Av. 15.2	24.8	36.1

Extensive tests with samples of various lots of dehydrated fish gave standard deviations of 1 pound for samples of the order of 10 pounds' toughness and 4 pounds for samples measuring 50 pounds. From these data it may be inferred that the average

toughness value of 10 pounds to 6 samples will be measured with a precision of less than  $\pm 5\%$ . Table I is an example of the data obtained under actual operation.

Organoleptic tests for texture changes made parallel with tests on the instrument have shown that the samples are placed in the same order in relation one to another but that small differences shown by the instrument are not always detected organoleptically.

This instrument is not designed to evaluate the quality of an unknown sample of dehydrated fish, but is of value in following the change in texture that occurs on storage. While no tests have been made on products other than dehydrated fish, it might be adapted for use with other such dehydrated products where alteration in texture during storage is a problem.

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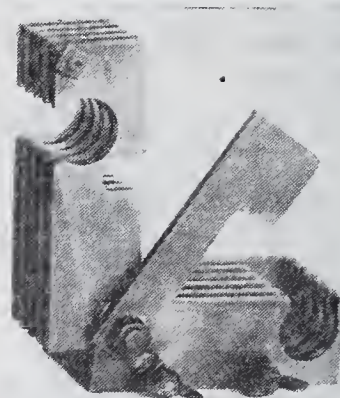


Figure 2. Shearing Jaws



# A General Utility Laboratory Distillation Column

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ANY articles have been written on the design of laboratory distillation columns, but the construction details of a practical column, suitable for general distillation operations, are not easily available to those not familiar with this field. The column described in this paper is believed to embody most of

the characteristics desirable for general utility. It is easily constructed of commonly available materials, suitable for most distillation operations over a wide temperature range, and free from mechanical or thermal strains. The essential feature is a glass heating jacket in which any desired distillation tube up to 45-mm. outside diameter may be inserted. There is also shown (Figure 1) a still head which is suitable for almost every type of distillation operation encountered in the laboratory. Its applications are discussed briefly.

## CONSTRUCTION

**JACKET.** The jacket, which is electrically heated, is made up of two concentric glass tubes, *G* 55 and *F* 70 mm. in outside diameter, held in place loosely by grooves cut in the two  $\frac{3}{4}$ -inch transite disks, *B*. These disks are bolted rigidly to a framework of three  $\frac{3}{8}$ -inch steel pipes, so that there is no mechanical strain on the glass. This arrangement allows both tubes to expand or contract independently of each other upon heating and cooling. The pipe framework is made rigid by means of two transite collars, *C*, fastened intermediate to the disks by setscrews. The inner glass tube is wound with two double-spiral, 20-ohm heating elements, *D*, so that the temperature of the upper and lower halves of the jacket may be adjusted independently. The heating elements are wound directly on the glass and held in place by moistened alundum cement. The ends of the elements are fastened securely by wire bands. The temperature of the jacket is measured by two thermocouples, *E*, placed inside the 55-mm. tube with the hot junctions located one fourth and three fourths of the distance along the jacket. The thermocouples are preferably strung as single strands running the length of the jacket. The electrical connections are made to the inner surfaces of the two end disks and the wires strung through the pipe framework.

**DISTILLATION TUBES.** The distillation tubes, which may be as large as 45 mm. in outside diameter, are inserted through the opening in the upper transite disk and rest upon the tapered opening of the bottom disk. The tubes smaller than 45 mm. in outside diameter are centered in the upper disk by means of split transite collars, *J*, and are provided with a ring of glass at the lower end if they are smaller than the bottom opening. (As an alternative arrangement the top of the distillation tube may be provided with a ring of glass which rests on the upper opening.) This arrangement, intended for use with standard-taper joints, allows the tube assembly to rise and pivot about the top when the bottom accessories are being attached. Breakage is thus prevented when the pieces are not correctly aligned. The distillation flasks are joined to the column by suspending them in a clamp attached at *A*, so as to raise the tube off the tapered opening in the flask by its own weight.

**STILL HEAD.** A still head, which has been found suitable for many operations, is constructed so that it may be inserted in the top of the distillation tube without clamping, thus facilitating the assembling of the bottom accessories. The head is constructed of a straight tube 25 mm. in outside diameter and is provided with vapor, *O*, and liquid, *L*, sampling lines and also a line, *K*, for returning liquid to the column. The inner tube of sample condenser in the vapor line should be constructed of 8-mm. tubing in order to prevent a liquid leg from forming in the condenser. The vapor line, together with its stopcock, and that portion of the head below the sampling lines are provided with a 30-ohm heating element. The windings on the vapor line and stopcock are wound over asbestos paper and coated with moistened alundum cement. The rest of the windings are wound directly on the glass. A thermocouple is wound around the barrel of the stopcock to measure the temperature at that point. A capillary thermocouple well, *M*, extending through the still head and, in the case of a packed column, down through the packing, may be used to measure the temperature at all points in the column.

## DISCUSSION

The column described above may be used for practically all types of distillation operations which are conducted above

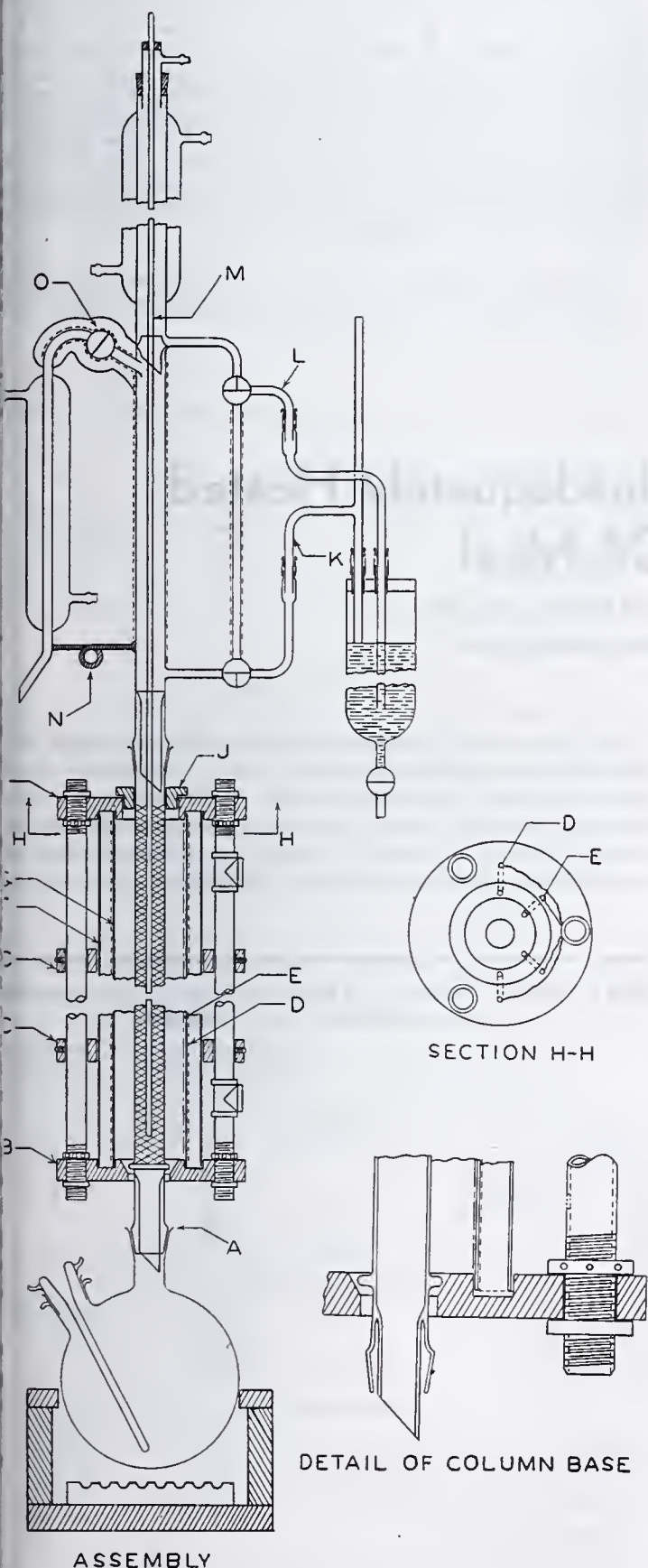


Figure 1. Diagram of Column

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room temperature and at pressures varying from a few millimeters of mercury absolute to several pounds per square inch gage. It is suitable for the analogous operations of absorption and extraction and may also be used as a reaction tube. In the latter case, the reaction tube should be provided with an additional heating element. While the design of this column is best suited for operations above room temperature, it may also be used for low-temperature work where it is necessary to observe the column action. In this operation, the cooling fluid would be circulated in the annular space between the 55- and 70-mm. jacket tubes. If the fluid was introduced several inches below the upper disk, only the lower end of the jacket would have to be sealed by a gasket.

The still head shown in the figure is a composite of several types used by the authors. The thermocouple well extending down through the packing is useful where the temperature is a criterion of the product compositions. While the thermocouple well will decrease the efficiency of the packing, this is in many cases compensated for by the gain in operability of the column. It enables the optimum reflux ratio to be set with a minimum of trial and error and the future course of batch distillations to be predicted without frequent readings of the temperature.

The vapor sampling line, *O*, is used where it is necessary to maintain a high reflux ratio and low holdup, as in the case of analytical distillations. Reflux ratios of 20/1 (*O/P*) are readily

and accurately obtained by adjusting the stopcock and then applying a slight pressure to the column (or vacuum to the receiver). The take-off rate is closely proportional to the square root of the pressure applied. Other advantages of vapor sampling are that it minimizes contamination of the sample by the stopcock grease and allows accurate sampling of distillates which give two liquid phases upon condensation.

A simple method of attaching accessories to the still head is illustrated in the figure for operation at total reflux with product holdup.

The receiver is suspended in a clamp, so that line *L* may be connected to it. The receiver is then rotated on its own axis until line *K* may be connected. The connecting lines have sufficient flexibility to allow them to be slid easily into place at the same time providing tight seals for vacuum work. The connections illustrated are pieces of rolled rubber tubing to provide flexibility. In the case of high-boiling organic solvents, standard taper joints may be used at points *L* and *K*. The capacity of the receiver may be varied by the use of return lines of different lengths. This same receiver, by closing the stopcock in return line *K*, may be used for automatically discontinuing the removal of product in batch distillations.

The applications of this still head to the various types of azeotropic distillations with two-phase condensate are similar to those above and do not require description.

## Method for Detecting Inadequately Heated Soybean Oil Meal

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**Editor's Note.** Since receipt of this paper, a subcommittee of the Animal Nutrition Committee of the National Research Council has been appointed to study tests which might be applied to soybean oil meals to indicate the degree of heat treatment and to correlate it with biological efficiency. The committee is carrying on collaborative work with additional samples in order to check further the validity of the urease test.

THE high nutritive value of soybean oil meal for poultry and swine depends considerably upon the heat treatment used in its preparation. Adequate heat treatment improves the biological value of the proteins (2, 3, 4) and simultaneously inactivates the enzymes present (6). The enzyme lipoxidase if left active in the meal could readily cause the destruction of vitamin A or its precursors with which it comes in contact in the digestive tract of animals. The contemplated use of urea in feed mixtures for ruminants makes it important that the soybean oil meal used in such mixtures be heated sufficiently to inactivate urease. Mixtures of inadequately heated soybean meals or raw soybeans and urea develop the highly characteristic odor of ammonia and, hence, become unpalatable.

It has been reported by Bird and co-workers (1) that commercially produced meals differ markedly in their nutritive values when used as the principal source of protein in the chick ration. Some of the poor results obtained were attributed to the use of insufficiently heated meals. With the enormously increased production of soybeans and their subsequent conversion into meal by plants having no previous experience with this commodity, the need for a rapid test for determining adequacy of heat treatment is apparent.

Since the over-all processing conditions of temperature, time, and moisture content favorable for protein denaturation would also be favorable for the inactivation of enzymes, a test based upon the enzymatic activity of the finished product was indicated. Urease was selected because of its unusually high concentration in soybeans and the ease with which its presence could

Table I. Growth Response and Results of Tests on Samples of Meal Receiving Different Heat Treatments

Treatment	Average Chick Weight at 9 Weeks, Grams <sup>a</sup>	Results of Test Solution pH
Experiment I		
Raw beans	476	8.9
143° F., 11.6 minutes	528	8.8
173° F., 16.5 minutes	586	8.6
175° F., 10.5 minutes	572	8.7
217° F., 42 minutes	669	7.6
Solvent meal	798	7.1
Hydraulic meal B	637	8.9
Experiment II		
Insufficiently cooked <sup>b</sup>	664	8.4
Medium cooked <sup>b</sup>	728	7.1
Properly cooked <sup>b</sup>	733	7.1
Overcooked <sup>b</sup>	784	7.1
Experiment III <sup>c</sup>		
Raw meal	317	8.6
Autoclaved 2.5 minutes at 20 pounds	494	7.1
Autoclaved 7.5 minutes at 20 pounds	483	7.1
Autoclaved 12.5 minutes at 20 pounds	443	7.1
Autoclaved 60 minutes at 5 pounds	494	7.1

<sup>a</sup> Growth data from (1).

<sup>b</sup> Producer's designation.

<sup>c</sup> Weights given for 7th week.



Table II. Precision of Test

Date	Results on Test Solution, pH
May 27	7.59
August 2	7.68
August 9	7.72
August 10	7.55
August 11	7.59

detected. The following test based upon Sumner's (5) qualitative test for this enzyme was devised.

#### PROCEDURE

To approximately 10 ml. of 0.05 M phosphate buffer solution pH 7.0 containing 0.3 gram of urea and 2 drops of 0.1% phenol solution is added 0.2 gram of the meal under test. The mixture is allowed to stand with occasional shaking at 25° to 30° C. for 30 minutes. If sufficient urease is present to cause an increase in the pH of the solution of one unit as indicated by a change in color to deep red, the meal has not been heated sufficiently. Adequately heated meals produce little or no color change. If the presence of alkaline salts is suspected, a blank could be run, using a sample of the meal which has been inactivated by heating at 135° C. for 30 minutes.

Table III. Results Obtained on Samples of Commercially Produced Meals

Type	Brand	Samples Tested	Results on Test Solution
Hydraulic	A	7	pH 8.3
Hydraulic	B	12	pH 8.5
Hydraulic	C, D, E	16	No change
Solvent	H, L	5	No change
Expeller	A, F, G, I, J	45	No change

#### RESULTS

In order to correlate the chemical test with actual feeding value, samples of meals of known history were secured through the courtesy of H. R. Bird of the Maryland Experiment Station. The results obtained using this test on those samples are given in Table I. The readings are given in terms of pH values instead of color change because of the greater accuracy obtainable. In general, those meals which gave the poorest growth response inhibited the highest urease activity. Unfortunately, the test cannot be used to indicate excessive heat treatment.

A sample giving intermediate values was selected for periodic testing in order to determine the reproducibility of results. The results given in Table II were obtained with a Beckman pH meter and indicate a variation of not more than 0.2 pH unit, which is well within the range of the colorimetric method.

The results given in Table III show that some meals produced by the hydraulic process on the market today are insufficiently heated.

#### SUMMARY

A simple rapid test based upon the urease activity of the soybean oil meal has been devised to detect inadequately heated soybean meals.

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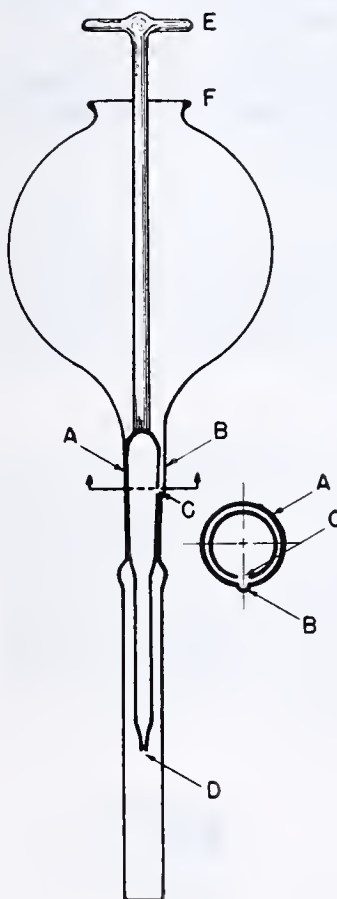
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## Dropping Funnel

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WORK for which a steam-jacketed dropping funnel is required the ordinary type is unsatisfactory because of difficulty in manipulating the stopcock to give a regulated flow of liquid from the funnel. It is also unsatisfactory where the liquid contained in the funnel must not dissolve stopcock grease.



The figure shows a dropping funnel which does not possess these difficulties. This funnel contains an internal ground joint, A. The liquid in the bulb flows down a narrow groove, B, in the outer wall and through a 2-mm. hole, C, in the hollow center plug (see enlarged cross-sectional view). This tapers down to a dropping point, D, which shows the rate of flow through opening C. On the upper lip of the top of the funnel is a small point of glass, F, and the handle, E, is placed in such a position that E and F are in the vertical plane with B and C when the hole is in the open position.

The entire funnel and ground joint can be placed in a steam or hot water bath, the ground seat needs no lubricant, for the contained liquid will so act, and if any liquid leaks through the joint it can drop only into the reaction flask.

#### ACKNOWLEDGMENT

The author wishes to thank Ray Newberry, university glassblower, for his work in constructing this apparatus.

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## Collective Index of Analytical Edition—Progress Report

Work on the fifteen-year cumulative index to the ANALYTICAL EDITION of INDUSTRIAL AND ENGINEERING CHEMISTRY, first announced in the July issue, has gone forward steadily, and it is now planned to send the index to the printer in November, so that distribution can be begun early in 1945.

The index will form a book about the same size as an issue of the ANALYTICAL EDITION, and will be printed on the same paper. It is being prepared by Charles L. Bernier, Associate Editor of *Chemical Abstracts*.

In the November 15 issue of the ANALYTICAL EDITION will appear a definite announcement of price and date of issue, with full instructions regarding placing of orders, for the benefit of those who have not yet done this.



# Electron Microscope Studies of Colloidal Carbon in Vulcanized Rubber

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New techniques are described for electron microscopy studies of colloidal carbon in vulcanized natural and synthetic rubber, by which it is hoped to make it possible to determine the micromorphology of carbon-reinforced rubbers, assess the effect of differences in carbon fineness and structure, evaluate visually the effect of polymer differences upon the ultimate carbon-polymer units, and determine the effect of processing and other variables.

**E**LECTRON microscope studies of carbon in vulcanized rubber have always been complicated by the difficulty of preparing specimens thin enough for penetration by the electron beam. The main attention has thus been given to investigations of carbons and rubbers separately.

## WORK ON CARBONS AND RUBBERS

Discussions of the particle size of the various carbons and their correlation to the physical properties of rubber compounds have been published by Wiegand and Ladd (6). Photomicrographs of natural and Perbunan latices were shown by von Ardenne and Beischer (1) in 1940. Morphological features of particles from latices of 16 plant species were studied by Hendricks, Wildman, and McMurdie (3). Photomicrographs and particle diameters for natural, Buna S, Buna N, neoprene, and Thiokol latices were published by Wiegand (5) in March, 1944.

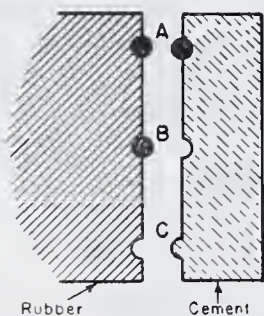


Figure 1

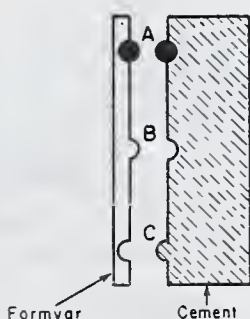


Figure 2

Other studies have been made on cements and fibers. Von Ardenne prepared films of rubber by spreading a thin film of latex on a glass slide, breaking the slide, and stretching the film. He also cast films from a solution of rubber in benzene (1). Studies on vulcanized and unvulcanized rubber have been carried out by Hall and co-workers (2) by allowing films cast from a cement to break into fibers and examining these fibers in the electron microscope.

**CONTEMPORARY WORK ON COMPOUNDS.** The earliest pictures of vulcanized, carbon-reinforced rubber were shown by von Ardenne (1) who prepared the specimen by crushing a sample of vulcanized rubber cooled by liquid air, and then choosing the finest fragment by means of a light microscope. Prebus (4) prepared rubber specimens by cutting a section of cable insulation by means of an abrasive wheel. The number of fragments suitable for electron microscope investigation made by either of these two methods is extremely small and therefore other means of preparation were desirable.

## DEVELOPMENT OF NEW METHODS

As a first attempt in investigating carbon-rubber specimens studies were made on uncured tread stock compounds made in cements from which thin films were cast. The rubber films were supported on collodion to prevent their breaking into fibers. These studies gave evidence of the ability of "structure" carbon to survive the shearing stress involved in milling in rubber (4). However, it was felt that dissolving the rubber compound, and then casting a film, changed the dispersion from that of the milled stock. Consequently other methods were sought. The methods have been developed:

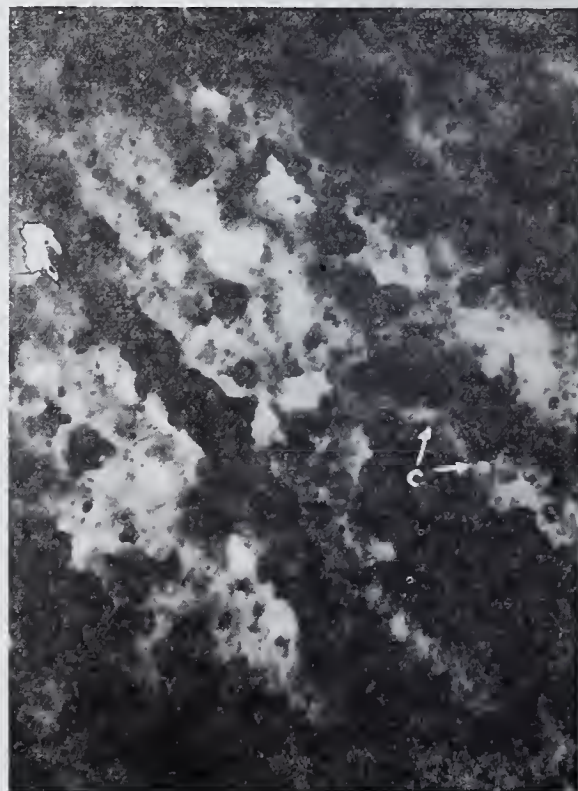
1. **RUB OUT TECHNIQUE.** In this method, a Formvar film first obtained on a glass microscope slide. A small quantity of uncured stock is then rubbed out on the Formvar by strokes of spatula or the edge of a glass slide. A 200-mesh screen is then cemented to the rubber smear by means of Ambroid around the edge. The Formvar film is teased free and floated on a water surface from which it is taken and allowed to dry.

The specimens can be examined in the electron microscope given a dry heat cure, and photographed again.

The disadvantages in this method are: Method of smearing such that streaking in one direction is produced; and dry heat cure is different from that employed in pressure molds.

2. **REPLICA METHOD.** This involves cracking a rubber block and obtaining a Formvar replica of the broken surface. The appearance of the carbon in the broken surface is analogous to that of stones in a broken concrete block.

A cured rebound block (2 × 1 × 1 inch) made of standard tread stock is first frozen in an acetone-dry ice bath, then placed in a vise and cracked into two pieces. First attempts to obtain



CRD-1

Figure 3. P-33 in GR-S (X 5000)



replica of the broken surface involved putting a Formvar film directly on the rubber. A suitable film for the electron microscope could not be stripped off, however. In order to overcome this difficulty, molten medium DeKhotinsky cement was poured on the surface (polystyrene could also be used). This was stripped off when hard and a drop of 2% Formvar solution in ethylene dichloride placed on the impression. After the film had hardened, the cement was dissolved in Solox. The Formvar replica obtained was then photographed.

The interpretation of the resultant photomicrograph requires a series of prints made at varying exposures. Three types of densities are representative of carbon particles in the original block.

**Particle A.** The original broken surface will have carbon particles protruding and holes where particles remaining in the other half of the block have been ripped out.

A protruding particle (A, Figure 1) may be ripped out by the cement. This will be carried directly into the Formvar film (A, Figure 2) when the cement is dissolved and will be a black circle on the print.

**Particle B.** A protruding particle (B, Figure 1) may remain in the rubber block and will be represented by a hole in the cement. This will give rise to a pimple on the Formvar film, and in the print will give a black circle lighter in density than that corresponding to particle A.

**Particle C.** A hole (C, Figure 1) in the block will be carried over to the Formvar as a hole. This will appear in the print as a white circle.

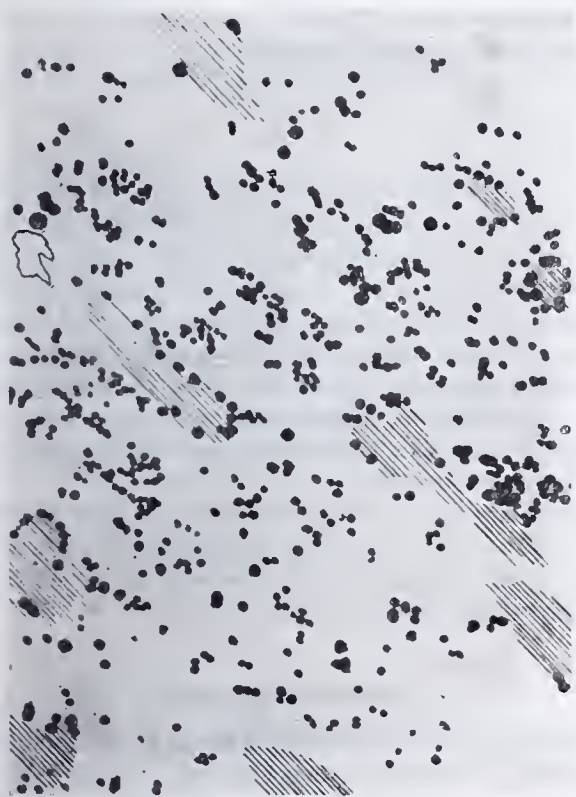


Figure 4. Dispersion of P-33 in GR-S Block

A photomicrograph of a replica from a block of P-33 in GR-S is shown in Figure 3, overexposed to bring out the white circles (particle C). Bits of rubber pulled out by the cement are also evident.

In Figure 4 is a map made from the various prints, showing the actual dispersion of P-33 in the GR-S block.

**3. VULCANIZING METHOD.** This method has been developed more than the preceding two, because of its greater efficiency. In principle it consists of pressing out the uncured stock to a thin film and then vulcanizing it.

The rubber is pressed out between two specially prepared disks 0.25 inch in diameter (Figure 5). Disk A is pressed out of 1/16 inch steel plate with a die shaped to give a crown. Filing the one side flat gives the shape shown in Figure 5. Disk B is flat and pressed out of 1/16 inch aluminum. The inner faces of both

A and B are coated with collodion, which serves a twofold purpose: it fills any holes in the metallic surfaces and thus allows slippage of the rubber as the pressure is applied, and it enables the extremely thin film to be removed from the mold. The piece of rubber tread stock placed between the two disks is less than 1/32 inch in diameter.

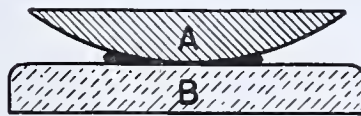


Figure 5. Disks

Three sets of disks with rubber between them are placed between two 6-inch square flat mold plates. In Figure 6 one of the sets has been left separated to show the piece of rubber in position. The mold is then placed in a Carver press, a pressure of 1000 to 4000 pounds per square inch is

applied, and the specimens are cured.

After curing is complete, the disks are separated and placed in amyl acetate. This dissolves the collodion and the pieces of thin film are teased free, allowed to remain in the amyl acetate for several days, then picked up on 200-mesh screens, and photographed in the electron microscope.

#### PHOTOMICROGRAPHS

Photomicrographs of specimens of vulcanized rubber prepared by the above method are shown in Figure 7. The compounds were as follows:

Micronex W-6 in GR-S	
GR-S	100.0
Micronex W-6 (EPC)	50.0
Zinc oxide	3.0
Bardol	7.5
Benzothiazyl sulfenamide	1.2
Sulfur	1.8
Micronex W-6 in Natural Rubber	
Smoked sheets	100.0
Micronex W-6 (EPC)	50.0
Zinc oxide	3.0
Stearic acid	4.0
Pine tar	2.0
BLE	1.5
Sulfur	2.7
MBT	0.9
P-33 in GR-S	
GR-S	100.0
P-33 (FT)	50.0
Zinc oxide	5.0
Bardol	4.5
Pine tar	3.0
Sulfur	1.8
Benzothiazyl sulfenamide	1.0

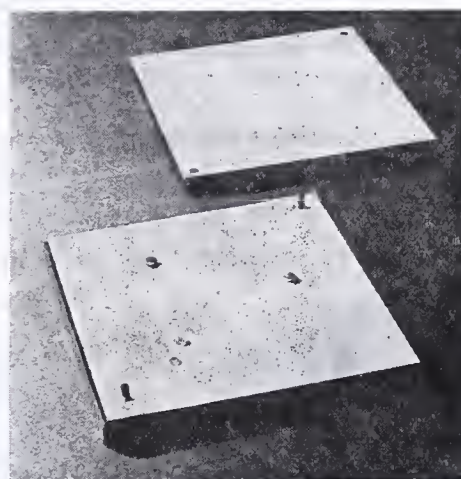
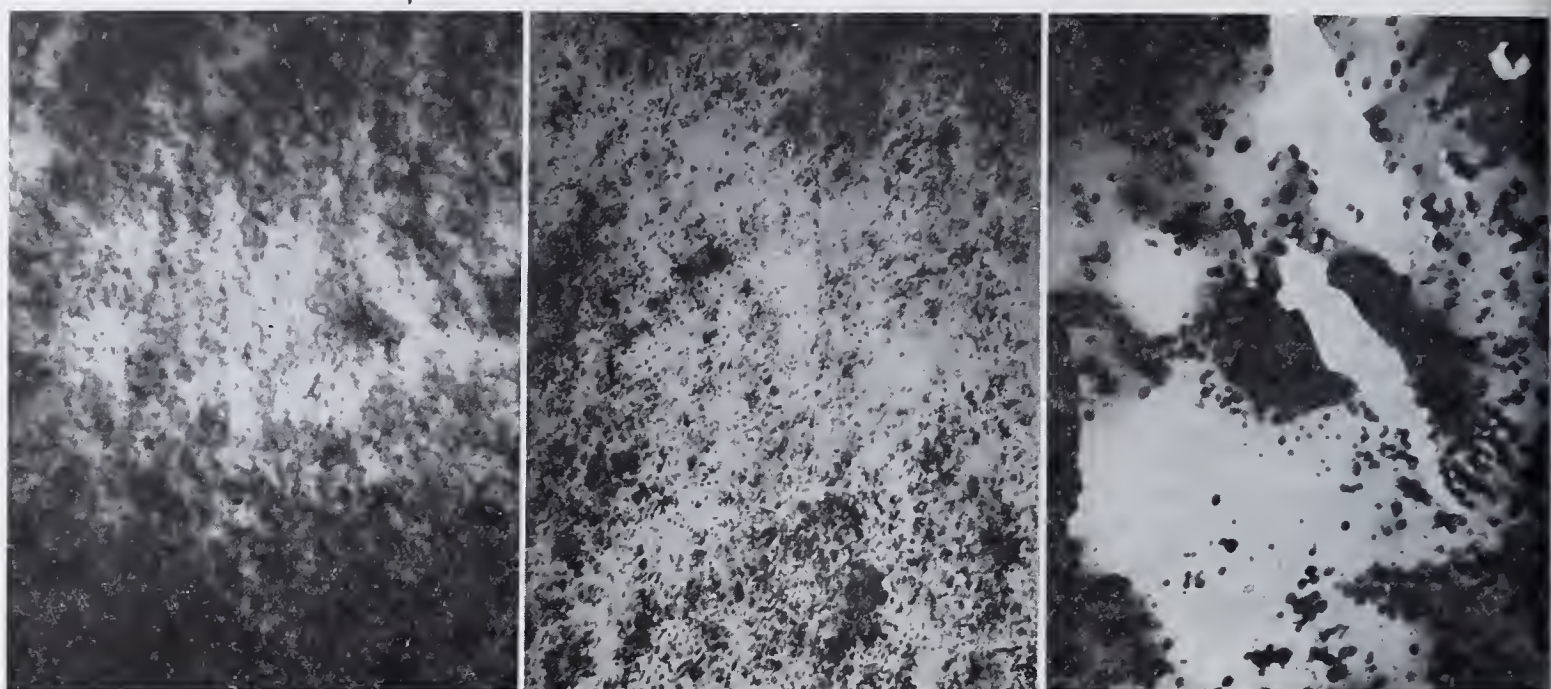


Figure 6. Mold Plates

Several tests were made to check on whether the carbon was being squeezed out of the rubber onto the collodion and might become insoluble in amyl acetate during curing. One test consisted of taking stereoscopic pictures. These reveal the carbon to be inside the rubber film. Figure 8 presents a pair of stereoscopic pictures, which show interesting tears. The density at A indicates a thickness of rubber equal to that of the P-33 particle. The serrated edge at B matches the carbon particles at C. The





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Figure 7. Photomicrographs of Vulcanized Rubber Specimens (X 5000)  
 Left. Micronex W-6 in GR-S    Center. Micronex W-6 in natural rubber    Right. P-33 in GR-S

radius of curvature has increased, indicating a contraction of the rubber after tearing. The weakness of the bond between the P-33 particle and the rubber is also shown by the cleanness of the break.

#### DISCUSSION OF METHODS

The rub out technique, because of its directional effect, is poorest of the three methods.

The vulcanizing method is the most successful of the three, and gives the best pictures. Some distortion may be present due to the high pressures used.

The replica method involves no distortion of the rubber block. Its disadvantage lies in difficulty of interpretation of the pictures and poor definition in the case of replicas of the fine carbons.

Studies now being made involve use of the last two methods.

#### DISCUSSION OF RESULTS

The definitive analysis of the disposition of reinforcing carbon particles in natural, and even more importantly in synthetic

rubbers, is a problem the solution of which is recognized as cardinal. It is hoped that the new techniques here described may in due course result in pictures from which it may be possible (a) to determine the micromorphology of carbon-reinforced rubbers; (b) to assess the effect of differences in carbon fineness and structure; (c) to evaluate visually the effect of polymer differences, as in gel content, changes due to heat exposure, latex particle size, etc., upon the ultimate carbon-polymer units; and (d) to determine the effect of processing and other variables in the carbon-polymer network.

Any who are in a position to furnish specimens embodying variables (c) and (d) in a strictly controlled series, are invited to correspond with the author, with a view to such electron microscopic analysis as opportunity may afford.

#### ACKNOWLEDGMENTS

Grateful acknowledgment is made to W. B. Wiegand, Director of Research, Columbian Carbon Company, for his kind interest and suggestions. Acknowledgment is made to E. R. Gilliland, Assistant Rubber Director, for permission to publish at this time.

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Figure 8. Stereoscopic Pictures of P-33 in GR-S



# Device for Projecting an Image of a Reading Scale

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SINCE the optical system now in use in these laboratories to facilitate the reading of microchemical balances has proved satisfactory, the authors believe a description might be of value to others engaged in similar work.

For routine analyses, a number of Kuhlmann microchemical balances are used, in which the pointer passes across a white scale with black line indexes at 0.2-mm. intervals. Deflection of the pointer is estimated to a tenth of one of these divisions—an operation obviously impossible for the unaided eye. Ordinarily a monocular telescope magnifier giving 5X or 6X magnification is directed at the scale. Operators who make many daily readings in this manner suffer considerable eyestrain and fatigue in making precise observations and would therefore find desirable a projected scale image that could easily be observed with both eyes.

It appears impossible to provide sufficient light on the conventional white scale to make possible its projection with adequate brightness and sufficient magnification. A light source of wattage high enough to provide sufficient light cannot be used inside or even in proximity to the balance case because of the danger of setting up convection air currents.

The authors have solved the problem by replacing the white ivory scale with a highly efficient specular reflecting surface. For this purpose they selected a piece of optically polished stainless steel and upon its surface reproduced a replica of the microbalance scale by means of a technique worked out for the photographic reproduction of reticles. Mechanical engraving of the lines and subsequent filling in with black pigment would serve as well, but would probably be more expensive.

The steel mirror surface reflects about 80% of the incident light, most of which is utilized, instead of scattering it inefficiently as does the matte white scale. The optical system actually employed in directing light to the scale and receiving it for projecting a magnified scale image might well be of various forms and dimensions, depending upon the particular apparatus to which it is applied. The system used in the microbalances is shown diagrammatically in Figures 1 and 2.

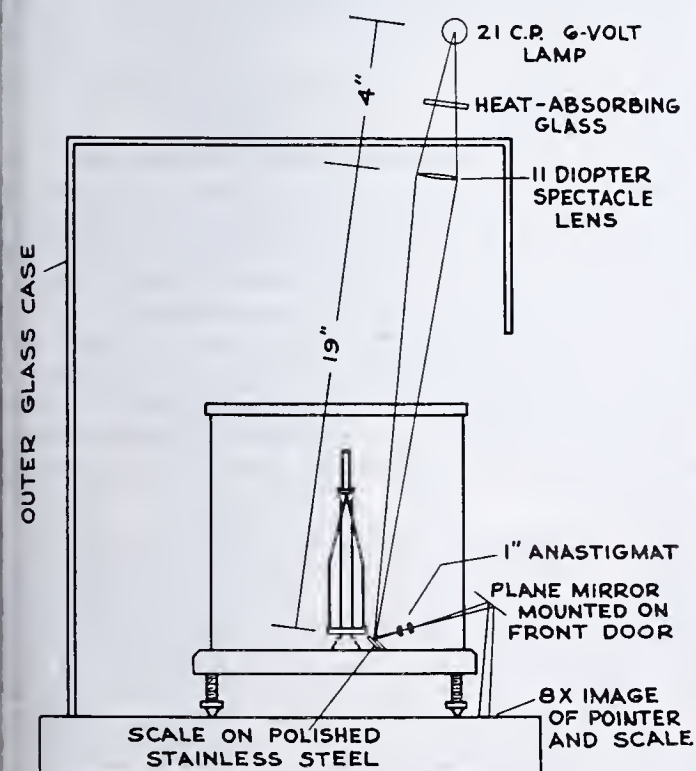


Figure 1. Microbalance with Scale Projecting System

In use, the balances are set up inside glass cabinets that protect the instruments from air currents and mechanical injury. The lamp in a housing is placed outside this outer cabinet. The filament of the lamp is imaged at about 5X magnification slightly above the stainless steel mirror. This slightly out-of-focus filament image produces acceptably uniform illumination on the mirror. The scale reflects the image into a 2.5-cm. (1-inch) focal length objective which projects the scale image and the image of the shadow of the pointer onto a white screen at a magnification of about 8X. For convenience, the image is directed downward by means of a small plane mirror, so that it falls on the bench in front of the instrument in a position which can be conveniently seen by the operator. The image is bright enough to be clearly visible in a well-lighted room. The illumination level is about 20 foot-candles.

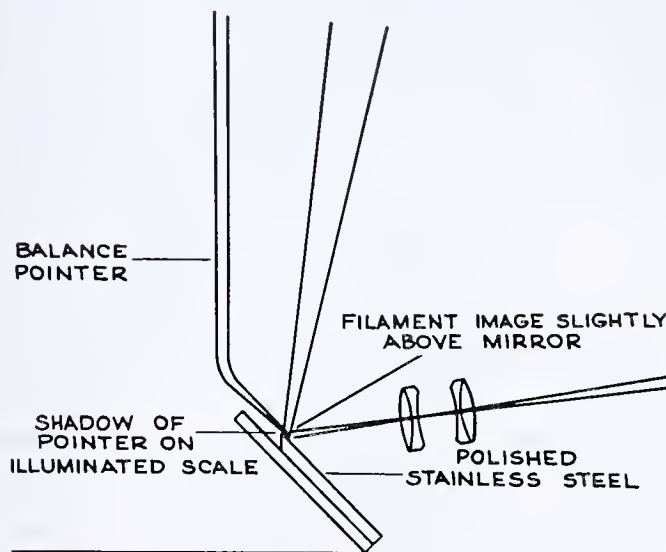


Figure 2. Side View of Pointer Scale, and Projection Objective

Since it is important to avoid heat within the balance case, it is desirable to determine how much energy is absorbed inside the case. The energy radiated by the lamp and accepted by the first lens amounts to about 0.2 watt, 80% of which is absorbed by the heat-absorbing glass (Corning Aklo). About 0.04 watt is the amount of energy reaching the balance scale. Almost 30% of this energy is reflected by the mirror surface through the lens and about 70%, or less than 0.03 watt, goes to heat the instrument. This is less than the radiant energy entering the case from other light sources in the room. Prolonged trial shows no heating effect upon the delicate mechanism of the balances.

COMMUNICATION 979 from the Kodak Research Laboratories.

## Spectrographic Boron Steel Standards

The National Bureau of Standards, Washington, D. C., is prepared to furnish six samples of boron steels in rod form for spectrographic standards.

The standard samples are cylindrical rods  $7/32$  inch in diameter and 4 inches long. The 4-inch rod may be cut at the center, giving two rods each 2 inches long for use as self-electrodes. The price per sample is \$3.00.

No.	Kind	Total Boron, %
425	Mn-Ni-Cr (N.E. 9450)	0.0006
426	Cr-Mo (SAE 4150)	0.0011
427	Cr-Mo (SAE 4150)	0.0027
428	Mn-Cr	0.0059
429	Ni-Cr-B	0.0091
430	Ni-Cr-B	0.019



# Determination of Iron in Food Products

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A modified thiocyanate procedure in which the color complex is extracted with isobutyl alcohol is described. Data on recoveries of added iron to a wide variety of foods are given. Recoveries of better than  $\pm 0.5$  p.p.m. are possible.

IRON content is an important factor in the nutritional evaluation of foods. As a trace contaminant promoting oxidative and metallic flavors, rancidity, and vitamin instability it also plays an important role. A satisfactory method for the determination of iron should be adaptable to a wide variety of products without alteration of procedure and should have greater sensitivity than the methods now used for food and biological materials. Some foods having a very low iron content, but a high phosphorus and calcium content, present a particularly difficult problem. Interference by both calcium and phosphorus is encountered with the relatively large sample required to provide a significant amount of iron. Increasing the sample size of such a food proportionately increases the amount of calcium and phosphorus. A typical example of such a food is powdered whole milk. The proposed method is sufficiently sensitive to measure small quantities of iron, and the interference of both calcium and phosphorus is negligible.

A review of the literature reveals a rather confusing, and in some cases contradictory, mass of information on the use of the thiocyanate reagent in the determination of iron. Thiocyanate is particularly well suited for use on samples prepared by acid digestion (wet-ashed) with a resulting high acid concentration and, for this reason, was chosen for the reagent. According to Woods and Mellon (8) the following variables must be kept reasonably constant: (1) amount and kind of acid, (2) excess quantity of oxidizing agent, (3) time of standing, (4) presence and amount of certain interfering ions, and (5) dielectric constant of the solvent.

Pyrophosphates resulting from dry-ashing interfere with the determination by complexing the iron (8). This interference can be eliminated by digesting the sample in sulfuric, nitric, and perchloric acids. Jackson (4) used acid digestion satisfactorily, and has shown that excellent recoveries can be obtained. Acid digestion is particularly advantageous where copper is also to be determined, as both determinations can be made from a single sample preparation.

Most foods may be readily ashed by digestion with sulfuric, nitric, and perchloric acids. However, a few products of high fat content do not lend themselves readily to complete oxidation. Dairy products are particularly difficult to digest, and at best, their sample preparation is a tedious, time-consuming procedure. Thirty per cent hydrogen peroxide has been used to excellent advantage in the oxidation of both high fat and carbohydrate products (5). The alternate ashing procedure described under the sample preparation has reduced the time for the preparation of a 5-gram sample of powdered whole milk to less than one half of that required when peroxide is not used.

Hallinan (3) has shown that a high concentration of hydrochloric acid is exceptionally well suited for the development of the ferric thiocyanate complex and that a high concentration of potassium thiocyanate gives the maximum color development in the presence of a minimum concentration of iron. A concentration of 1.5 *N* hydrochloric acid and 0.5 *N* potassium thiocyanate in the presence of potassium persulfate as an oxidizing agent gives excellent color development and stability. Potassium persulfate may be used satisfactorily as an oxidizing agent both to

ensure that iron remains as the ferric ion and to stabilize the color complex in aqueous solution. Although potassium persulfate has been reported to develop a yellow color in hydrochloric acid solution (8), this has not been noted in this study. The comparative stability of the ferric thiocyanate complex with and without added persulfate is shown in Table I.

Table I. Comparative Stability of Ferric Thiocyanate Complex the Aqueous Phase

Time of Standing Min.	Transmission	
	No potassium persulfate added %	Potassium persulfate added (1 ml. of 2% solution) %
0 (initial reading)	50.2	50.4
5	51.0	50.3
10	52.2	50.4
15	52.8	50.3
20	53.8	50.4
35	55.5	50.5
45	56.8	50.9
60	59.4	51.0

Isobutyl alcohol appears to be an ideal solvent for concentration and intensifying the color, and a satisfactory means of eliminating the interference of calcium. The ferric thiocyanate complex extracted from an acidified aqueous solution with isobutyl alcohol has a maximum absorption at 485 millimicrons, obeys Beer's law (Figure 1), and is free from the variations which occur when isobutyl alcohol is used (2). The point of maximum absorption was determined using a Coleman Universal spectrophotometer and checked with a Beckman quartz spectrophotometer. Winslow (7) has shown in his study of the intensity and stability of the complex that the dielectric constant is a satisfactory criterion for the dissociation of the solute. Isobutyl alcohol has a relatively low dielectric constant (International Critical Tables value of 18.7) and the resulting solution of the complex is stable for several hours. Although isobutyl alcohol is fairly soluble in water it can be effectively used if the volume ratio of the aqueous phase to the alcohol is kept constant. A ratio of 41 ml. to 25 ml. was selected, since this allows aliquot sizes up to 25 ml. and gives ample color intensification.

Calcium is probably the most difficult interfering ion. It has been reported (2) that when it is present in excess of 10 mg. per 100 ml. of a hydrochloric acid solution, the quantitative recovery of iron cannot be obtained. As it is present in substantial quantities in dairy products, recovery data were obtained on iron added to these products. The data were obtained by both the author and a cooperating laboratory (6) and are presented in Tables II and III. The recoveries indicate that calcium interference, if any, is negligible when the proposed method is applied to high calcium content foods. In addition to obtaining recovery data, the amount of calcium extracted by isobutyl alcohol was determined.

Seventy milligrams of calcium were dissolved in 25 ml. of water. The solution was treated as a blank and extracted according to the proposed method. The calcium content of the isobutyl alcohol following the extraction was found to be less than 0.2 mg. An additional test was made to determine the amount of hydrochloric acid that was extracted by the isobutyl alcohol. A blank determination containing only water, hydrochloric acid, thiocyanate, and isobutyl alcohol was made. The initial concentration of acid in the aqueous phase was found to be 1.3 *N*. Following the usual 2-minute shaking period, the normality had dropped to 0.96. This loss of acid by absorption by the isobutyl alcohol increases the normality of the solvent to approximately 0.55 *N*.



The presence of a relatively high concentration of hydrochloric acid in the isobutyl alcohol undoubtedly aids in the stabilization of the ferric thiocyanate complex.

The stability of the ferric thiocyanate complex has been checked on several determinations by reading the per cent transmissions immediately and then again over periods ranging up to 36 hours. In no cases were increases in transmittancies noted. One sample with an initial reading of 23% was read again at the end of 36 hours. The transmission was found to be 26% or an increase of only 3%. However, recovered isobutyl alcohol previously used for either thiamine or iron determinations resulted in rapid fading and hence could not be used satisfactorily.

A single distillation from an all-glass Pyrex still frees isobutyl alcohol from its initial iron content and, inasmuch as it is a standard solvent for thiamine assays, it is readily available in food laboratories doing vitamin analyses.

#### METHOD APPLICATION

**APPARATUS.** A spectrophotometer or photoelectric colorimeter (a Coleman Universal spectrophotometer was used by the author). Readings are made at 485 millimicrons, the point of maximum absorption.

**Pyrex glassware.** All glassware is cleaned with concentrated nitric acid, rinsed with distilled water, and finally rinsed several times with redistilled water.

**REAGENTS.** Sulfuric acid, concentrated, reagent grade. Nitric acid, concentrated, reagent grade, redistilled from Pyrex. Perchloric acid, double-vacuum-distilled 72% perchloric acid may be obtained from G. Frederick Smith Chemical Co., Columbus, Ohio).

Hydrogen peroxide, 30%, without added stabilizer. Redistilled water, distilled water redistilled from Pyrex. Hydrochloric acid, concentrated, reagent grade. Potassium persulfate, reagent grade, 2% solution in redistilled water, prepared fresh every few days and stored in a refrigerator. Potassium thiocyanate, reagent grade, 20% solution in redistilled water prepared frequently and stored in a refrigerator. Isobutyl alcohol, b.p. 106–107° C., redistilled from Pyrex.

**Standard Iron Solutions.** Stock Solution. Weigh exactly 0.0000 gram of iron wire into a dry, iron-free beaker. Dissolve in 10% hydrochloric acid to which 1 to 2 ml. of concentrated nitric acid have been added. Carefully evaporate to dryness and dissolve in the minimum amount of hydrochloric acid. Transfer quantitatively to a 1000-ml. volumetric flask and dilute to volume. This stock solution contains 1 mg. of iron per ml.

**Working Standard.** Dilute 10 ml. of the stock solution to 100 ml., adding a few drops of bromine water just prior to adjusting to volume. This solution contains 10 micrograms of iron per ml.

**SAMPLE PREPARATION.** Transfer an accurately weighed sample (3 to 5 grams, depending on the suspected iron content) to a 300-ml. Kjeldahl flask, add 10 ml. of nitric acid, and warm slightly to start oxidation. When the initial oxidation has subsided, add 2 ml. of concentrated sulfuric acid and boil gently until charring commences. Prepare liquid samples by taking appropriate volumes, depending on the iron content, and concentrating to a small volume in the presence of 10 ml. of nitric acid before the sulfuric acid is added.

Add nitric acid, a few milliliters at a time, or preferably, dropwise until the oxidation is nearly completed as evidenced by only slight darkening upon evolution of sulfur trioxide fumes. Remove the flame and allow the flask to cool slightly. Add 1 ml. of perchloric acid and continue heating until the solution has clarified. It may be necessary to add a few more drops of nitric acid at this point to complete the oxidation. Heat to the point where copious white fumes of sulfur trioxide appear and the perchloric acid has been destroyed. The final solution should be colorless, or, at most, a light straw color. Cool, add 40 ml. of redistilled water, and boil until copious white fumes of sulfur trioxide again appear. Continue heating for about 5 minutes to assure complete oxidation and elimination of perchloric and nitric acids. Cool, add about 10 ml. of redistilled water, and quantitatively transfer to a 100-ml. volumetric flask by washing with small portions of redistilled water until the volume is nearly 100 ml. Cool to room temperature and dilute to volume.

**ALTERNATE METHOD OF SAMPLE PREPARATION.** This method is particularly advantageous for foods of high fat content.

Transfer an accurately weighed sample to a 300-ml. Kjeldahl flask, add 5 ml. of 30% hydrogen peroxide and 2 ml. of concentrated sulfuric acid, and heat gently until charring commences.

Add 5 ml. more of the 30% hydrogen peroxide and continue heating until charring again occurs. Proceed as in the regular method, beginning with the addition of the nitric acid, a few milliliters at a time.

**PROCEDURE.** Transfer a 25-ml. aliquot of the prepared solution to a 125-ml. separatory funnel and add exactly 5 ml. of concentrated hydrochloric acid. Add 1 ml. of 2% potassium persulfate and swirl the separatory funnel to ensure complete mixing. Add exactly 10 ml. of the 20% potassium thiocyanate reagent to develop the color, then add exactly 25 ml. of isobutyl alcohol and shake for 2 minutes. Draw off and discard the aqueous layer. Invert and slowly revolve the funnel to dislodge any water particles clinging to the walls and allow to stand for 10 minutes. Draw off the small amount of water which has separated from the alcohol, and transfer the alcohol layer to a dry 50-ml. Erlenmeyer flask. Immediately prior to reading the % transmission add a small amount (about 0.1 gram) of anhydrous sodium sulfate and agitate, to remove suspended particles of water from the alcohol extract. Read at 485 millimicrons, setting a reagent blank at 100% transmission. Obtain the micrograms of iron from a standard curve and convert to p.p.m.

If the color is too intense to read (in excess of 50 micrograms) repeat the determination, using a smaller aliquot of the prepared sample. As it is important that the volume ratio be kept constant, the difference in the aliquot size must be made up by the addition of redistilled water—for example, if a 15-ml. aliquot is used in place of the usual 25-ml., correct the difference in volume by adding 10 ml. of redistilled water.

**PREPARATION OF STANDARD CURVE.** Develop the color on increments of the working standard in the range of 0 to 60 micrograms of iron. A convenient formula to follow is:

$$\begin{aligned} &5 \text{ ml. of concentrated hydrochloric acid} \\ &x \text{ ml. of standard} \\ &(25 - x) \text{ ml. of redistilled water} \end{aligned}$$

From this point proceed exactly as outlined in the method, beginning with the addition of the potassium persulfate. Plot per cent transmission against the concentration on semilogarithmic paper.

Table II. Analyses of Foods

Product	Iron Content	Iron <sup>a</sup> Added	Total Iron Calculated	Total Iron Found
	P.p.m.	P.p.m.	P.p.m.	P.p.m.
Whole-kernel yellow corn	5.7	6.0	11.7	11.7
Peas	19.1	6.0	25.1	25.4
Tomatoes	6.5	6.5	13.0	12.5
Lima beans	14.6	3.7	18.3	18.2
Blackberry jam with added guava	7.3	5.5	12.8	12.5
Sliced bacon	7.3	4.8	12.1	11.9
Pork sausage meat	15.1	3.8	18.9	18.8
Wheat and soy egg noodles	39.7	10.0	49.7	49.8
Flour, enriched	27.7	10.0	37.7	37.7
Rice, converted, samples representing various stages in milling process				
Sample A	3.4	10.0	13.4	13.5
Sample B	8.8	10.0	18.8	18.8
Sample C	13.2	15.0	28.2	28.3
Sample D	16.5	10.0	26.5	26.5
Sample E	45.9	15.0	60.5	60.7
Patent flour, unenriched <sup>b</sup>	8.3	10.0	18.3	18.2
Patent flour, enriched with ferrum reductum <sup>b</sup>	30.6	5.0	35.6	35.5
Bread made from enriched flour <sup>b</sup>	32.8	5.0	37.8	37.7
Whole wheat flour <sup>b</sup>	37.3	10.0	47.3	47.5
Bread made from whole wheat flour <sup>b</sup>	39.5	5.0	44.5	44.5
Powdered whole milk	4.0	4.0	8.0	8.0
Powdered whole milk	4.1	6.0	10.1	10.6
Evaporated milk	4.0	2.0	6.0	6.0

<sup>a</sup> Iron added prior to digestion of sample.

<sup>b</sup> Submitted by Methods Committee of American Association of Cereal Chemists.

#### DISCUSSION

Contamination by fly ash so prevalent in industrial areas may be reduced to a minimum by acid digestion of the samples in Kjeldahl flasks. Thus the chief sources of contamination in the proposed method lie in the reagents. Sulfuric acid is the most frequent offender, but occasional lots of potassium thiocyanate and anhydrous sodium sulfate have proved unsatisfactory. In all cases reagent blanks should be run to determine the quality of new reagents. The author has found that, even in cases of reagent contamination, exact control of all volumes makes it possible to obtain satisfactory recoveries. In one instance, a badly



contaminated lot of sulfuric acid resulted in a blank of 75% transmission when compared to isobutyl alcohol set at 100%, but several points were checked on the standard curve with good agreement. Volumetric transfer pipets should be used throughout the method, so that the iron contamination due to a reagent will be kept absolutely constant throughout the blanks and the determinations. The addition of the redistilled nitric acid during the sample preparation need not be critically controlled, as this reagent will be iron-free.

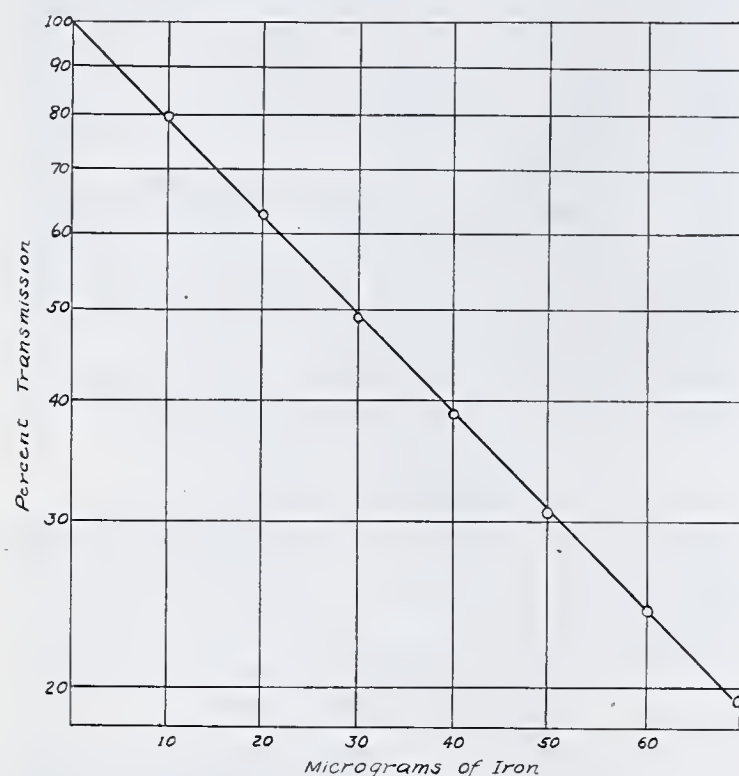


Figure 1. Standard Curve

The separation of the last traces of water from the isobutyl alcohol must be carefully controlled. Following the first separation the ratio of alcohol to water becomes very large, and if at this point more than gentle revolving of the flask is used a slight emulsion may occur. This will result in slow separation of water and may produce erratic results. Centrifuging will correct this difficulty. However, if the method is closely followed, clean separations may be easily obtained.

The quantity of anhydrous sodium sulfate added to the final extraction should be only sufficient to clarify the alcohol solution of suspended water. If more than the specified amount is added, the equilibrium between the dissolved water and the alcohol may be upset, resulting in the separation of a substantial portion of the dissolved water. With a few preliminary trials an analyst can easily estimate the amount to be added when using a small spatula, or, preferably, a scoopula.

The proposed method has proved entirely satisfactory when applied to a wide variety of food products (Table II). The recoveries have been better than  $\pm 0.5$  p.p.m. As a further test of its reliability it has been submitted to three laboratories for additional checking and verification. One laboratory working with cereal products did not submit data obtained by the method but commented that it gave results in good agreement with values obtained by other methods (1). Two laboratories applied it to products that offer particularly difficult problems—beer with high phosphorus content and spray-dried whole milk and nonfat dry milk solids representing high-calcium foods. The results submitted by these two laboratories are shown in Table III.

The sensitivity of the method is exhibited by data obtained using two types of instruments. When the Coleman Universal spectrophotometer with 13-mm. cuvettes is used, the maximum range is from 0 to 70 micrograms of iron (Figure 1), representing a desirable working range of 3 to 50 micrograms. O'Malley (6) reports that using a Pfaltz and Bauer fluorophotometer as a filter photometer the maximum range was found to be from 0 to 30 micrograms of iron. This instrument uses a cuvette with a greater transmission path. The sensitivity of the Coleman Universal spectrophotometer may be correspondingly increased by using the larger cuvettes and the special cuvette carrier available for this instrument, as the volume of the isobutyl alcohol extract is sufficiently large to be read in the larger cuvettes. For general work the 13-mm. cuvettes are very satisfactory, as a wide range of iron concentration can be covered. For very low concentration where small samples are prepared, it is advantageous to use the larger cuvettes.

Table III. Analyses of Foods by Cooperating Laboratories

Product	Iron Content P.p.m.	Iron Added <sup>a</sup> P.p.m.	Total Iron Cal- culated P.p.m.	Total Iron Found P.p.m.
Dry whole milk (spray) <sup>b</sup>				
Sample 1	3.5	8.0	11.5	12.2
Sample 2	1.8	8.0	9.8	9.2
Sample 3	6.8	10.0	16.8	16.4
Sample 4	3.3	8.0	11.3	10.8
Sample 5	7.6	..	..	..
Sample 5 (duplicate determination)	7.2	..	..	..
Sample 6	6.6	..	..	..
Sample 6 (duplicate determination)	6.4	..	..	..
Sample 7	4.0	..	..	..
Sample 7 (duplicate determination)	4.8	..	..	..
Nonfat dry milk solids (spray) <sup>b</sup>				
Sample 1	4.2	8.0	12.2	12.0
Sample 2	6.8	..	..	..
Sample 2 (duplicate determination)	7.2	..	..	..
Sample 3	6.8	..	..	..
Sample 3 (duplicate determination)	6.8	..	..	..
Beer <sup>c</sup>				
Sample A	0.24	0.80	1.04	1.04
Sample B	0.22	0.80	1.02	1.12
Sample C	0.22	0.80	1.02	1.08
Sample D	1.22	0.80	2.02	2.00
Sample E	0.38	0.80	1.18	1.10
Sample F	0.20	0.80	1.00	1.00

<sup>a</sup> Iron added prior to digestion of sample.

<sup>b</sup> Data submitted by American Dry Milk Institute, Laboratory Department, Chicago, Ill.

<sup>c</sup> Data submitted by Continental Can Co., Research Department, Chicago, Ill.

#### ACKNOWLEDGMENT

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# Determining Ascorbic Acid in Large Numbers of Plant Samples

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A procedure for the determination of ascorbic acid in plant material is described which has been found useful in plant breeding and other plant research. It allows the rapid determination of ascorbic acid in a large number of samples of plant material with a satisfactory degree of accuracy.

IN THE course of plant breeding experiments the author desired to know the ascorbic acid content of many plants at different stages of development. Available methods for the determination of this substance in plants were too time-consuming to assure a reliable comparison of values from different sources inasmuch as changes might occur in the tissue while awaiting analysis. Hence it seemed imperative to devise a method which could permit the assay of many samples in the shortest possible time. This paper describes a procedure which has enabled the author and three student assistants to make 500 assays for ascorbic acid per day and to compare the results thus obtained with those of published methods.

It was obvious from early experiments that a more effective and rapid means of disintegrating plant tissue than hand grinding was necessary in order to gain speed in the assay as a whole. Hence a mechanical device (Figure 1) which makes possible the simultaneous disintegration of ten samples within 2 to 6 minutes, depending upon the texture of the tissue, was designed for the purpose and constructed in the machine shop of Michigan State College.

## GRINDING MACHINE

As shown by Figure 1 this machine consists of a metal frame which supports a row of ten metal seats, each holding a porcelain pestle 75 mm. high and 45 mm. in diameter. Porcelain pestles are attached to tapered chucks similar to those used in drill presses and are driven from a common horizontal shaft by means of belts and quarter-turn pulleys. Pressure on the pestle shafts can be varied by changing the position of the weights on the horizontal levers shown. Additional pressure can be obtained by holding down the levers singly or simultaneously with a cross bar.

While this investigation was in progress, Morell (7) published a method which possessed certain advantages but was still in-

adequate for the author's purpose. Morell made use of the so-called Waring Blendor for the maceration of plant samples. The efficiency of this Blendor was compared with that of the grinding machine described. The Blendor permits the use of much larger samples, and therefore reduces the error, but this advantage is lost if very small samples have to be disintegrated. Furthermore, the Blendor is ineffective for macerating certain types of plant material, such as seeds and fibrous tissue. These difficulties could not be overcome by the use of smaller Blendor cups as described by Davis (3) and Benne (1).

## ASSAY

The Waring Blendor was used in most cases, but where it did not work well with a particular tissue the grinding machine was used.

A new method of handling very large samples, especially if a number of leafy plants should be examined together, has been successfully tested. A small portion of a sample is placed in the container with the liquid necessary for the extraction, and the machine is operated long enough to obtain a homogeneous mixture. The mixer is stopped, another part of the sample is added, and the procedure is continued until the entire sample has been disintegrated. In such a manner, voluminous samples weighing over 100 grams can be analyzed, but the extracts are rather viscous, and the filtration is therefore slow. If the extract appears to be too viscous, the amount of liquid should be increased. From the standpoint of accuracy a large sample is preferred.

If the grinder is used, the weight of samples should not exceed 5 grams.

The titration is carried out with a rather concentrated solution of 2,6-dichlorophenolindophenol using a microburet. Flat dishes of glazed porcelain or sillimanite have been found extremely useful as containers. The small amount of extract is placed in their inner rim by means of a suitable pipet. The dish is held at an angle of approximately 60° (Figure 2) and gently rotated in the same plane through a short arc. By this means the liquid is moved enough to ensure its immediate mixture with the dye which is added drop by drop from the microburet. This is a very convenient procedure because the titration is completed rapidly and the end point is seen much more clearly than in any other way.

REAGENTS. Metaphosphoric acid, 20% stock solution. Dissolve 200 grams of metaphosphoric acid sticks in cold distilled water, filter, and dilute to 1 liter. Use one part of the stock solution and 9 parts of distilled water for extractions in the

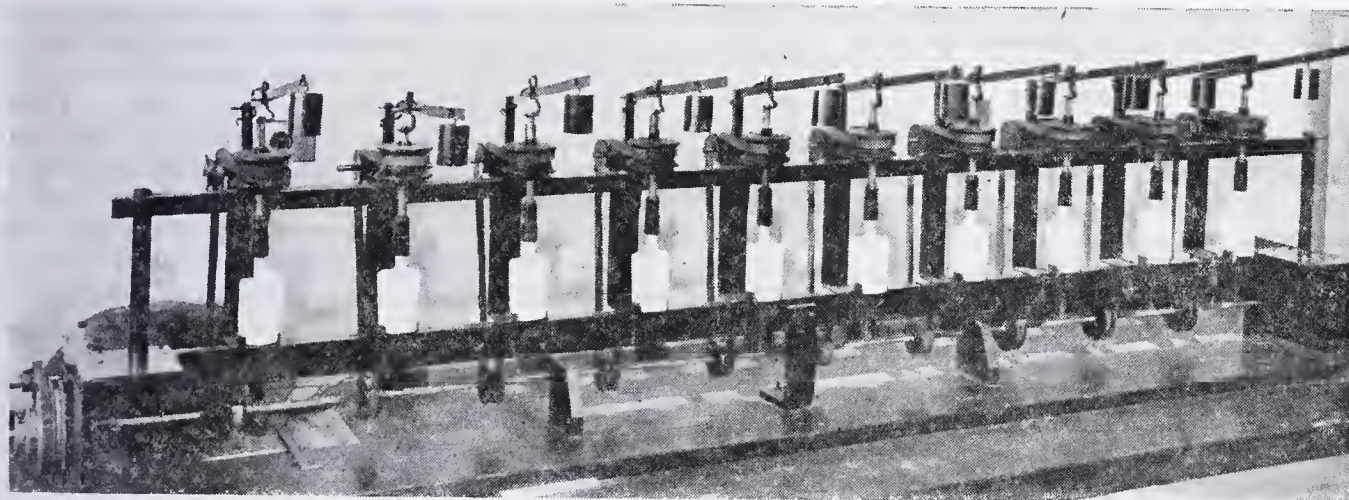


Figure 1. Grinding Machine for Simultaneous Disintegration of Ten Samples



**Table I. Stability of 2% Metaphosphoric Acid Solution Stored at 4°**

(As shown by ascorbic acid values of extracts)

HPO <sub>3</sub> Stored Days	Ascorbic Acid Found		
	Tomato juice, home made	Cauliflower, Purple Head, "curd"	Mustard, Tendergreen, leaves
	<i>Mg. per 100 grams of material used</i>		
0	12	67	166
5	13	67	166
10	12	67	167
15	12	67	162
20	12	67	165
30	12	67	160
40	11	64	148

**Table II. Stability of Sodium 2,6-Dichlorobenzenoneindophenol in Aqueous Solution at 4°**

[As compared by standardization after Menaker and Guerrant (5), expressed as percentage of value obtained on first day]

0	5 days	10 days	15 days	20 days	25 days
100	99.7	100	100	96.5	94.3

**Table III. Ascorbic Acid in Fresh Plant Material**

Plant Material	Ascorbic Acid Found <sup>a</sup>		
	Waring Blendor	Grinding machine	Hand grinding
	<i>Mg. per 100 grams of fresh material</i>		
Cabbage, wrapper leaves	81 ± 0.21 <sup>b</sup>	81 ± 0.21	80 ± 0.15
Cauliflower, "curd"	89 ± 0.17	88 ± 0.19	92 ± 0.32
Gladiolus, leaves	316 ± 0.86	342 ± 1.38	322 ± 0.90
Mustard, leaves	156 ± 0.87	149 ± 0.86	150 ± 0.86
Pepper, green fruit	161 ± 0.45	161 ± 0.61	157 ± 0.82
Tomato, fruit	25 ± 0.38	24 ± 0.36	26 ± 0.38

<sup>a</sup> Means of 25 determinations.

<sup>b</sup> Standard deviation of mean.

mixer (2% solution). All concentrations can be stored in the refrigerator at 0° to 5° C. for 30 days (Table I).

Sodium 2,6-dichlorobenzenoneindophenol solution. Dissolve 200 mg. in 100 ml. of warm distilled water, filter, and dilute to 1 liter. When stored in the refrigerator the solution remains usable for at least 2 weeks (Table II).

**PROCEDURE WITH BLENDOR.** One hundred milliliters of 2% metaphosphoric acid are pipetted into the dry glass container of the Blendor. The material to be examined (10 to more than 100 grams) is weighed to the nearest 0.1 gram, as suggested by Morell (7), and placed in the acid. The Blendor is operated for 2 to 5 minutes, depending on the kind and quantity of plant material. The liquid is filtered through qualitative filter paper (Green Nos. 488 and 704 were used in these experiments) into a 125-ml. Erlenmeyer flask. Aliquots of 0.1 to 5 ml. depending on the expected ascorbic acid content of the extract, are taken by pipet and titrated.

**PROCEDURE WITH GRINDING MACHINE (SEMIMICROMETHOD).** Three milliliters of 20% metaphosphoric acid are pipetted into the grinding cups. The samples (different tissues or duplicates, as desired), weighed accurately to the second decimal, are placed in the cups, and a small amount of quartz sand is added. The samples are ground for 2 to 6 minutes, 27 ml. of distilled water are added to bring the solution to a concentration of approximately 2% metaphosphoric acid and the mixture is stirred briefly and gently and filtered through qualitative paper.

#### DISCUSSION

Ascorbic acid concentration in samples of various plant material as obtained by different methods of maceration was determined on a large scale (Table III). In all instances, when firm tissue was ground, the results of machine grinding were higher than those of hand grinding, which may be explained by the fact that hand grinding, when performed over a longer period, is tiresome and thus lowers efficiency. This is evident from the table inasmuch as cabbage, gladiolus, and pepper represented samples with comparatively firm tissue. Hand grinding gave lower yields of ascorbic acid in these cases if it was compared with the average of both machine grinding methods. In the case of gladiolus the Waring Blendor did not work satisfactorily, however, since the blades did not completely disintegrate the fibrous leaves.

In the comparison of the different ways of disintegrating plant material (Table III) it was necessary to use samples of different sizes according to the means of maceration employed. The samples prepared for the Blendor weighing 20 grams, those to be ground 2 grams. The small samples were selected as representative portions from the large ones, which were then reduced to the weight of 20 grams.

**FILTRATION VERSUS CENTRIFUGATION.** Centrifuging samples as used in the standard method, slows up the process. It has been proved by numerous comparative tests that no losses of any significance occur if the extract to be tested is filtered through paper instead of being centrifuged. An extensive survey is given in Table IV on the basis of 500 tests made with various plant material. The plant tissue was disintegrated in the Waring Blendor and half of the extract was filtered through folded filter Green No. 488 while the other half was centrifuged for 10 minutes. The saving of time is clearly indicated by the fact that enough filtrate was available for titration in the average case within 3 minutes, whereas it took nearly 15 minutes to bring a centrifuged sample to that point. The results justify the abandonment of centrifugation in favor of filtration.



**Figure 2. Glazed White Dishes for Microtitrations**

**TITRATION.** Comprehensive comparisons were made between titration and colorimetric determination as developed by Mindel and Butler (6) and Bessey (2) and used in many later modifications, as, for instance, the one proposed by Morell (7). The colorimetric determinations were made by means of a Coleman Universal spectrophotometer at the laboratory of the Department of Foods and Nutrition of Michigan State College and a Color photometer at the Section of Agricultural Chemistry, Michigan Agricultural Experiment Station. The plant samples were macerated in the Waring Blendor, and the extract was divided in half after filtration and examined immediately. The results are given in Table V. In some instances a significant difference appears to exist between the results obtained. However, higher readings were obtained in approximately as many cases with one method as with the other. It is concluded, therefore, that titration can, under certain conditions, well replace the more time-consuming colorimetric determination.

After the termination of these studies Loeffler and Pontig (4) published their adaptation of the photometric method



Table IV. Ascorbic Acid in Filtered and Centrifuged Plant Extracts

Plant Material	Ascorbic Acid Found <sup>a</sup>	
	Filtered Mg./100 g. of material used	Centrifuged
Basswood, leaves (July)	70 ± 0.43 <sup>b</sup>	71 ± 0.45
Beans, string, fresh		
Variety 1	16 ± 0.30	16 ± 0.36
Variety 2	10 ± 0.21	10 ± 0.23
Variety 3	10 ± 0.13	10 ± 0.15
Variety 4	26 ± 0.30	26 ± 0.26
Variety 5	15 ± 0.23	15 ± 0.21
Beans, string, dehydrated		
Variety 6	16 ± 0.15	16 ± 0.10
Variety 7	9 ± 0.27	9 ± 0.22
Variety 8	9 ± 0.18	9 ± 0.13
Variety 9	17 ± 0.23	17 ± 0.18
Variety 10	38 ± 0.35	38 ± 0.20
Cabbage, heads		
Variety 1	47 ± 0.18	48 ± 0.29
Variety 2	38 ± 0.22	38 ± 0.13
Variety 3	32 ± 0.22	34 ± 0.18
Variety 4	46 ± 0.20	47 ± 0.22
Variety 5	37 ± 0.23	37 ± 0.16
Variety 6	37 ± 0.16	36 ± 0.15
Variety 7	33 ± 0.16	34 ± 0.16
Variety 8	46 ± 0.22	46 ± 0.22
Variety 9	50 ± 0.21	50 ± 0.15
Cabbage, leaves of flowering plant		
Sample 1	129 ± 0.43	129 ± 0.43
Sample 2	162 ± 0.33	159 ± 0.87
Cauliflower, "curd"		
Sample 1	77 ± 0.23	77 ± 0.23
Sample 2	103 ± 0.50	103 ± 0.53
Cauliflower, leaves	74 ± 0.58	74 ± 0.58
Dandelion, flowers	21 ± 0.30	21 ± 0.33
Kohlrabi, edible portion	75 ± 0.23	75 ± 0.22
Kohlrabi, leaves	146 ± 0.65	145 ± 0.53
Mustard leaves (grown in greenhouse)		
Sample 1	76 ± 0.40	75 ± 0.34
Sample 2	73 ± 0.29	72 ± 0.38
Sample 3	65 ± 0.23	65 ± 0.31
Sample 4	48 ± 0.35	49 ± 0.16
Sample 5	99 ± 0.37	98 ± 0.34
Pepper, green fruit		
Sample 1	157 ± 1.08	160 ± 1.09
Sample 2	257 ± 1.65	257 ± 1.66
Pepper, leaves		
Sample 1	98 ± 0.81	98 ± 0.76
Sample 2	167 ± 1.48	167 ± 1.37
Radish, tops (grown in greenhouse)	82 ± 0.55	80 ± 0.31
Red maple, blossoms	155 ± 1.61	156 ± 1.50
Ribes aureum, blossoms	61 ± 0.62	60 ± 0.57
Tomato, fruit		
Variety 1	17 ± 0.37	17 ± 0.54
Variety 2	23 ± 0.42	23 ± 0.51
Variety 3	31 ± 0.38	30 ± 0.27
Variety 4	35 ± 0.33	35 ± 0.25
Variety 5	29 ± 0.34	28 ± 0.26
Variety 6	28 ± 0.39	29 ± 0.39
Variety 7	19 ± 0.31	19 ± 0.20
Variety 8	24 ± 0.20	24 ± 0.15
Variety 9	25 ± 0.26	24 ± 0.21
Variety 10	23 ± 0.27	24 ± 0.31

<sup>a</sup> Means of 10 determinations.  
<sup>b</sup> Standard deviation of mean.

ascorbic acid determination. They emphasized the fact that "some analysts still use the erratic titration with an unbuffered acid" and mentioned 5% sulfuric acid plus 2% metaphosphoric acid in particular, as used by Mack and Tressler. They showed graphically the rate of fading of the indophenol reagent as caused by various acids in the absence of ascorbic acid. Although the diagram indicated that metaphosphoric acid does not cause much fading, it was considered appropriate to examine the conditions which exist during titration with an unbuffered acid.

Solutions of ascorbic acid in 1, 2, 3, 5, and 10% metaphosphoric acid, in 5% sulfuric plus 2% metaphosphoric acid, and in 0.4% oxalic acid, as recommended by Loeffler and Ponting (4), were prepared and tested (Table VI). Three kinds of plant material, tomato fruits, tomato leaves, and leaf lettuce, were extracted with equal amounts of 1, 2, 3, 5, and 10% metaphosphoric acid, 5% sulfuric acid plus 2% metaphosphoric acid, and 0.4% oxalic acid. The plant material was cut in small pieces, mixed thoroughly, and then divided in equal portions which were blended with the different acid solutions in a Waring Blendor. Table VII shows the ascorbic acid values found in these samples; 5 and 10% metaphosphoric acid caused some fading of the dye but the only serious case was represented by sulfuric plus metaphosphoric acid where a very considerable fading occurred. This confirms Loeffler and Ponting's findings. For

unknown reasons there was much less interference of the strong acids when certain plant material was used. The conclusion can be drawn that 1 to 3% metaphosphoric acid can be used for titration with no loss of ascorbic acid due to insufficient extraction and without the danger of destruction of the dye by the extracting acid.

In addition, the action of several concentrations of metaphosphoric acid on the indophenol dye was observed. Fading of the dye could be seen in 10% metaphosphoric acid if the solutions were mixed and allowed to stand for 2 to 3 minutes. However, after 3 minutes the degree of fading was not sufficient to cause an error of more than 3% in titration of ascorbic acid present in the metaphosphoric acid. This is immaterial in view of the fact that titrations as used in the procedure described require no more than an average of one minute each. Furthermore, much weaker concentrations of metaphosphoric acid are used in titrations with dichlorobenzeneindophenol.

It is concluded that titrations as used in the procedure presented can be accomplished safely and reliably with unbuffered solutions.

ACKNOWLEDGMENTS

Acknowledgment is due to G. D. Sherman, Department of Chemistry, Hawaii Agricultural Experiment Station, Honolulu,

Table V. Ascorbic Acid Values of Fresh Plant Materials

Plant Material	Ascorbic Acid Found <sup>a</sup>	
	Titration Mg./100 g. of material used	Colorimetry
Cabbage, Golden Acre, sections of head	30.9 ± 0.39 <sup>b</sup>	30.5 ± 0.01
Cabbage, Golden Acre, wrapper leaves	81.7 ± 0.16	82.0 ± 0.09
Cabbage, Wisconsin Hollander, sections of head		
Sample 1	36.6 ± 0.13	38.2 ± 0.07
Sample 2	46.2 ± 0.09	45.1 ± 0.01
Sample 3	37.1 ± 0.09	50.3 ± 0.12
Sample 4	42.0 ± 0.10	44.1 ± 0.01
Cabbage, Wisconsin Hollander, wrapper leaves		
Sample 1	92.6 ± 0.07	92.7 ± 0.01
Sample 2	91.8 ± 0.08	89.4 ± 0.01
Sample 3	95.5 ± 0.06	97.6 ± 0.01
Sample 4	99.1 ± 0.07	93.1 ± 0.01
Pepper, Harris King of the North, green fruit		
Sample 1	127.6 ± 0.10	123.6 ± 0.01
Sample 2	156.9 ± 0.12	162.5 ± 0.10
Sample 3	141.4 ± 0.14	144.0 ± 0.02
Sample 4	138.2 ± 0.12	141.4 ± 0.12
Gladiolus, Orange King, leaves		
Sample 1	374.9 ± 0.21	360.7 ± 0.54
Sample 2	323.1 ± 0.62	312.2 ± 0.35
Sample 3	327.4 ± 0.32	335.9 ± 0.30

<sup>a</sup> Means of 10 determinations.  
<sup>b</sup> Standard deviation of mean.

Table VI. Titration Values of Ascorbic Acid Dissolved in Metaphosphoric and Other Acids

Acid Concentration	Ascorbic Acid Values <sup>a</sup>
1% HPO <sub>3</sub>	100
2% HPO <sub>3</sub>	100
3% HPO <sub>3</sub>	100
5% HPO <sub>3</sub>	102
10% HPO <sub>3</sub>	104
5% H <sub>2</sub> SO <sub>4</sub> + 2% HPO <sub>3</sub>	125
0.4% (COOH) <sub>2</sub>	100

<sup>a</sup> Expressed as percentage of value obtained in 2% HPO<sub>3</sub>.

Table VII. Titration Values of Ascorbic Acid

Extracting Acid	Greenhouse Tomatoes (Immature Fruits)	Tomato Leaves	Greenhouse Leaf Lettuce <sup>a</sup>
	Mg. per 100 grams of material used		
1% HPO <sub>3</sub>	12	22	12
2% HPO <sub>3</sub>	12	22	14
3% HPO <sub>3</sub>	12	21	13
5% HPO <sub>3</sub>	12	24	13
10% HPO <sub>3</sub>	13	25	13
5% H <sub>2</sub> SO <sub>4</sub> + 2% HPO <sub>3</sub>	13	28	15
0.4% (COOH) <sub>2</sub>	11	21	14

<sup>a</sup> Average values of two varieties.



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## BOOK REVIEW

**Laboratory Experiments in Biological Chemistry.** James B. Sumner and G. Fred Somers. 169 pp., 17 figures. Academic Press, Inc., New York, N. Y., 1944. Price, \$2.60.

This volume was written as a laboratory guide for a course given to students of biochemistry at Cornell University. In the words of the author, "it is intended to be . . . general and to provide fundamental training in laboratory biochemistry to students in any field of study". It consists for the most part of 252 exercises which are largely written in the imperative, and with a minimum of detail and discussion.

In examining this small volume, the reviewer was most impressed by the amount and diversity of material which was compressed in a small space. It was obviously not intended to cover every phase of biochemistry, being most deficient in clinical procedures. Short of the larger reference books, it gives the most diverse treatment of the chemical phases of the subject that the reviewer has seen.

The greater part of this book is devoted to instructions for qualitative testing of many types of biological materials. It even includes more or less systematic procedures for identifying unknown fats, carbohydrates, and proteins singly and in mixtures. From the standpoint of qualitative biochemistry alone, this little volume should find a useful place on the biochemist's bookshelf.

The quantitative aspects of the field have been treated much less completely than the qualitative. Many of the standard analytical procedures are given in sufficient detail for routine performance, and most common types of biochemical analysis are included. It is unfortunate that most of the quantitative methods are old ones which are widely used but do not represent the best analytical procedures available at present. In this respect, this book will not serve to advance the progress of quantitative analysis by biochemists. A large proportion of the methods given are those of Folin, in original or modified form. Phosphate determination is made by the old method of Fiske and Subbarow and the Allen modification. No mention is made of the very advantageous procedure of Berenblum and Chain. Similar omissions might be noted for most of the quantitative procedures.

An unusual feature of this book as compared with others of the same type is its emphasis on safety and proper practice. This is a field which has been largely neglected in college textbooks and in teaching, in spite of the example of industry, where safety has been a primary consideration in many instances. A stronger trend in this direction is greatly to be desired.

As is always to be expected in a first edition, some minor deficiencies and inaccuracies are occasionally apparent. On page 23, it is not at all clear how a student is to "observe the absorption band in the red at 635  $\mu$ ". No mention is made of the use of any dispersion device for the purpose. On page 87, the description of the xanthoproteic test does not include any indication of the group in the protein molecule which gives the reaction, except for the statement: "Benzene compounds react with nitric acid to form yellow nitro compounds. Picric acid is an example." The other protein tests uniformly list the group responsible for the test. The statement on page 12, that "centrifuge tubes that are heated for the purpose of drying them become very brittle and are likely to break while being centrifuged", must seem surprising to one familiar with glass behavior. It would certainly have been better to make the statement accurate than to pass the phenomenon of strain formation off as brittleness.

The treatment of electrometric determination of pH is too nearly complete to force the student to consult a better reference, but is inadequate as a complete explanation in itself. Similarly, the treatment of colorimetry and the "photoelectric colorimeter" leaves something to be desired. For example, the discussion of Beer's law on page 71 omits the very important fact that this law was derived for monochromatic light and is not applicable to polychromatic radiation. This omission is even more serious in the discussion on page 37, of the Duboscq colorimeter, which employs white light and approximates obedience to Beer's law over short ranges only. This fact was tacitly recognized in the statement "the concentration of the unknown cannot be determined accurately in many cases if it differs too greatly from the concentration of the standard". On page 73, the injunction to use test-tube type absorption cells which "are of uniform thickness all the way around and are perfectly round" will certainly discourage the conscientious student. Studies of hundreds of such tubes have failed to produce even one which meets the above specifications. It would have been more nearly in the realm of possibility to require that tubes be matched with respect to rotation and used in the positions in which such matches were found.

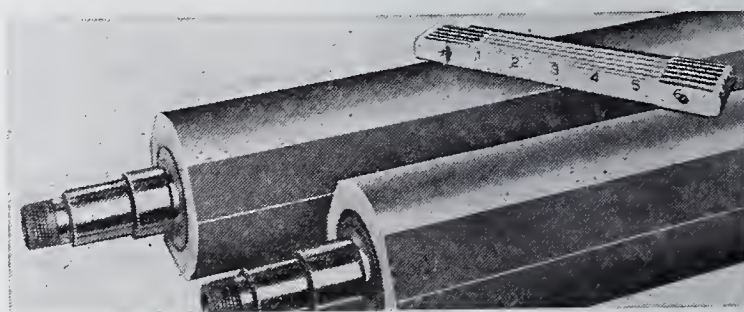
Typography, make-up, and binding of the book are very satisfactory. The printing is clear and remarkably free of typographical errors. In spite of the fact that this volume does not accomplish all that might be desired in a general laboratory manual, it is a valuable addition to the biochemical literature which can serve as the basis for a very effective laboratory course in biochemistry.

PAUL L. KIRK

## NEW EQUIPMENT

### Special Rolls for Laboratory Testing

Today's great expansion of industrial research has resulted in a demand for many types of rolls, specially designed for use in test processing of various materials.



The photograph shows a pair of experimental rolls recently made by the Rodney Hunt Machine Co., Orange, Mass. These rolls were built with an exceptionally soft rubber cover and were provided with three flat areas on the roll surface.



## Techniques of Quantitative Spectrographic Analysis

J. RAYNOR CHURCHILL, Aluminum Company of America, New Kensington, Pa.

SPECTROGRAPHIC methods are in many respects unique, since in no other method of determining composition are so many different fields of science and technology embraced. The preparation of the sample often entails problems in chemistry, metallurgy, and machining practices. The excitation of the spectrum may involve chemical reactions, thermodynamics, electronics, and atomic physics. The spectrograph itself introduces a wide variety of problems in optics and mechanical design. The photography and measurement of the spectrum involve a host of problems in photographic technique, organic and inorganic chemistry, temperature control, mechanical design, electricity, electronics, and optics. All of the widely varied problems encountered are closely related, and the variable factors in sampling, excitation, photography, and measurement are interdependent. Any problem in one phase of the spectrographic procedure necessarily involves all or most of the other phases of the spectrographic procedure as a whole. We cannot consider the excitation source without also considering the type of samples to be analyzed, the spectrograph, the photographic process, and the photometry of the spectra. We cannot discuss the photometry of spectrograms without reference to the materials analyzed, the excitation source, the spectrograph used, and the photographic procedure.

Because of the almost unlimited number of combinations and permutations of electrical, optical, chemical, and physical variables possible in spectrographic analysis and because of the interdependence of these variables on each other, there is no optimum value for any one of the variables except in relation to all of the others. Similarly, in comparing apparatus, it cannot be said that any one spectrograph, microphotometer, or source unit is superior. We must always think in terms of combinations of conditions, combinations of circumstances, and combinations of equipment. It is possible, in fact very frequent, that two widely different spectrographic procedures using dissimilar apparatus are found to be equally effective in the analysis of a particular material. Neither the specific procedural steps involved nor the instruments used are necessarily interchangeable individually between two such methods.

In the laboratories of Aluminum Company of America, specific techniques have been developed for use with particular combinations of equipment. It is fully recognized not only that widely different techniques might prove equally satisfactory with the same combinations of equipment, but that other combinations of equipment might be just as satisfactory. In the following discussion of spectrographic techniques, most of the material was

*The Analytical Edition of Industrial and Engineering Chemistry is concerned largely with the publication of papers submitted to it by authors, and emphasis is placed on original material. Authors naturally lay greater stress on the advantages rather than the disadvantages of their techniques and methods, and this sometimes results, especially in the field of instrumental analysis, in misconceptions on the part of those who are not specialists.*

*To help analytical chemists keep abreast of developments, with knowledge of limitations as well as advantages of various methods, invited papers will be printed from time to time, written by specialists in various fields. L. T. Hallett, associate editor, is devoting a considerable portion of his time to the development of such papers. These will not be reviews, but will be presented for the purpose of evaluating methods and apparatus as analytical tools and will emphasize the rigorous standardization and care that are often required in their use.*

*Three papers on spectrographic analysis, published in this issue, serve to introduce this new editorial policy: "Techniques of Quantitative Spectrographic Analysis" by J. Raynor Churchill, "Proposed Minimum Requirements for Emission Spectrographic Equipment Used in Quantitative Analysis" by Charles D. Guettel, and "Qualitative Spectrographic Analysis" by G. W. Standen.*

*Walter J. Murphy*, EDITOR



derived from the collective experience of the many spectrographic laboratories of Aluminum Company of America.

### EXCITATION

In devising a spectrographic procedure to meet a particular need, the first decision to be made is to select a suitable excitation source. The choice of a source unit depends upon the spectral response required—i.e., sensitivity of detection—the quantitative accuracy desired, the specific nature of the elements sought, and the characteristics, both physical and chemical, of the samples to be analyzed. The excitation sources ordinarily used in emission spectrography fall into three broad classes: direct current arcs, alternating current arcs, and alternating current sparks. Each has its advantages in particular applications and, since the important differences between the three classes are in degree and not in kind, there is a wide overlapping of the fields of use.

**DIRECT CURRENT ARC.** Until recently, the direct current arc has been the most widely used excitation source. Because of its high ultimate sensitivity and versatility of application, the direct current arc has been the general all-purpose source, without which the spectrographic laboratory was considered incomplete. Recent improvements in other sources and their use have altered this situation to such an extent that many modern spectrographic laboratories rarely use the direct current arc in quantitative metallurgical analysis, and in some specialized routine laboratories no arc facilities are provided.

The conventional direct current arc consists of a power source having an open circuit voltage of at least 220 volts and a variable resistance to regulate the current passing through the arc gap. The actual voltage drop across the gap is, of course, much lower than across the supply line and depends upon the current used, the resistance of the electrodes, and the resistance of the gap. Currents ranging from 3 to 10 amperes are generally used with metal electrodes, and currents ranging from 10 to 20 amperes are used with such nonvolatile materials as alumina, zirconia, or columbia. Metal samples are often used as self-electrodes in the arc when the melting point of the samples is sufficiently high. In most other cases the sample is placed in a cavity in a graphite rod which is used as the lower electrode. A second graphite rod is used as the upper electrode.

There are almost as many shapes of electrodes as there are laboratories using the arc. Lower electrodes vary from 0.125 to 0.3125 inch in diameter and vary in design from a simple rod containing a small cavity in the tip to the much more elaborate types providing capsules or platforms for holding the sample and constrictions to reduce heat loss. Simple electrodes are prepared on a motor-driven cutter, while the more complicated shapes are generally made on a lathe. The remarkable lack of unanimity among different laboratories in the choice of electrode dimensions and shapes seems to be caused partly by actual differences in analytical problems and partly by personal preferences and prejudices of spectrographers.

The direct current arc yields higher sensitivities of detection than most other sources for most of the elements detected by emission, and can be applied readily to almost any type of material. Most general qualitative analyses and many quantitative determinations of minute constituents, therefore, are best handled by the direct current arc. However, the general erraticness and poor reproducibility generally associated with the arc have prevented a wide application to the quantitative determination of any but minor constituents or trace elements. The disadvantages of this source in quantitative work arise largely from the fact that arc excitation is almost purely a thermal phenomenon. Any variable, such as gap spacing, electrode form, or matrix composition, which affects the amount of heat produced in the arc or the transfer of heat from the arc will affect the spectral intensities produced. When certain metals are present in the arc, oxides form rapidly and accumulate as a crust or beads, causing the arc to wander and sputter and to behave in a rather erratic manner. Also, the selective volatilization of the various constituents in the sample presents serious problems in controlling the discharge and in quantitative interpretation.

While the disadvantages discussed have largely excluded the direct current arc from most high constituent work other than qualitative or semiquantitative tests, it should not be inferred that these defects are insurmountable. Recent experiments in a number of laboratories have indicated that the objectionable effects usually associated with the direct current arc can be greatly reduced by the use of special types of electrodes and by the addition of fluxes or "buffers". Promising results on refractories, ore materials, and stainless steel samples have been obtained in several laboratories.

In the aluminum industry the direct current arc has been invaluable as a qualitative tool and has been applied to the determination of many minor constituents and trace elements. In most routine analysis on aluminum alloys, however, adequate response can be obtained with excitation sources of greater inherent stability than the direct current arc.

**ALTERNATING CURRENT ARC.** A more reproducible source than the direct current arc is available in the alternating current arc. This source, first described by Duffendack and Thompson (3), consists essentially of a 2000- to 4000-volt transformer equipped with a variable reactance or variable resistance, or both to control its output. Currents of 2 to 4 amperes are generally used with this apparatus.

The alternating current arc produces a somewhat more stable arc than the direct current arc at a small sacrifice of sensitivity of detection. As in the case of any other excitation source, the alternating current arc has a field in which its characteristic deficiencies are at a minimum. As a quantitative tool the alternating current arc has been applied most effectively in the analysis of caustic liquors and certain salts and in these applications its sensitivity rivals that of the direct current arc, and its reproducibility is much better. On metal samples the alternating current arc is in most cases superior to the direct current arc in reproducibility but inferior in sensitivity of detection. Most of the shortcomings of the direct current arc appear to a somewhat lower degree in the alternating current arc.

In the aluminum industry, the alternating current arc has been a valuable tool in laboratories primarily interested in secondary aluminum. In this application, it provides a rapid and economical means of obtaining a qualitative analysis combined with sufficiently accurate quantitative analysis for scrap sorting purposes. Among primary producers of aluminum, the alternating current arc has received little application because of a need for higher quantitative accuracy.

**ALTERNATING CURRENT SPARK.** At present most quantitative spectrographic analysis of metals is accomplished by means of excitation sources classified as spark units. Formerly a conventional spark unit consisted essentially of a 10,000- to 40,000-volt transformer, a capacitance of 0.002 to 0.02 mfd., an inductance of a few hundredths or a few tenths of a millihenry, and sometimes a resistance of a few ohms. The analysis gap, the self-inductance, and the resistance were used in series across the secondary of the transformer, with the capacitance connected in parallel with the gap. Spark units of this type were used in most metallurgical applications of the spectrograph until more refined excitation units became available.

The above-described type of spark unit is often referred to as an uncontrolled spark or as a free-running spark to distinguish it from spark units of more recent manufacture equipped with synchronous gaps, auxiliary gaps, tuned circuits, or inductive coupled quenching circuits. Figure 1 shows a schematic wiring diagram of the controlled spark in its most popular form, which differs from the uncontrolled spark only in the insertion of the synchronous gap developed by Feussner (4). The synchronous gap serves two purposes: (1) It serves as a timing switch, permitting the unit to produce only one discharge (one train of oscillations) per cycle or half cycle. (2) The additional synchronous gap or gaps in series with the analytical gap have a stabilizing effect. Much the same effect is produced by the use of a static



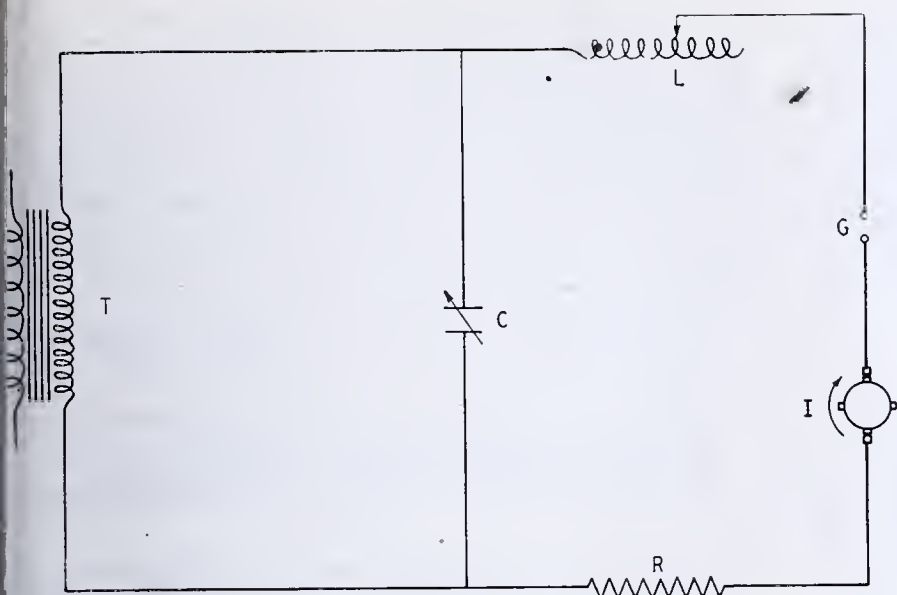


Figure 1. Typical Circuit for Controlled Spark Discharge

ary auxiliary spark gap subjected to a controlled air stream in place of the synchronous gap, although commercial models all employ the synchronous gap. Controlled spark units sometimes have a semifixed impedance in the primary which is more or less tuned with respect to the secondary to produce a condition approaching resonance. Other refinements, claimed to increase the stability of the discharge but not generally provided on commercial models, may include ultraviolet irradiation of either the analytical gap or the synchronous gap, or both, controlled air streams impinging on either or both gaps, or tuned quenching circuits inductively coupled with the spark circuit.

Controlled spark discharges, similar to those produced by the Feussner spark, have been produced by the use of electronic interrupters. Such a spark unit, developed for general spectrographic work, has been described by Malpica and Berry (8). Electronically controlled spark units have not been developed to the extent of those using mechanical interrupters and have received little application in industry. Because of possibilities of greater flexibility, more precise control, and elimination of moving parts, electronically controlled spark units may be expected to become increasingly important.

With the possible exception of new excitation units developed very recently, no other excitation unit has equaled the controlled spark unit in reproducibility and dependability in the quantitative analysis of metal samples. Accordingly, the spark unit has largely replaced both the direct current arc and the alternating current arc in most applications where its sensitivity of detection is sufficient and where there is a need for high precision. The chief limitations in the application of the spark lie in its low response as compared to the direct current arc or the alternating current arc, and the difficulties and inconveniences encountered in handling samples in certain physical forms, such as powders or solutions.

In connection with the high reproducibility of the controlled spark discharge, the literature contains frequent mention of standard deviations of 1% or less in repetitive analyses. While such claims are based on factual data and can be closely reproduced in most laboratories properly equipped for quantitative spark work, the standard deviations given should not be literally interpreted as measuring the expected accuracy of an analysis. These data are obtained on repetitive tests on the same sample, and the sample is generally specially selected for uniformity in order to remove all sampling effects in studying the discharge. Such tests yield valuable information as to precision or reproducibility on a particular sample but do not measure the actual effective precision of the analysis, nor even that portion of the

analytical error which should be properly ascribed to excitation. It has been found that spectral response in a spark source is affected by the following factors not taken into account by simple reproducibility tests reported in the literature:

- Composition of the matrix.
- Physical form of the electrodes.
- Differences in oxidation effects on samples and standards.
- Particle size of dispersed constituents in the sample.
- States of combination of the elements in the sample.
- Metallurgical history of metal samples.

The above factors enter into most routine analysis by spark methods and cause changes in excitation which in turn produce measurable errors in analysis. In routine practice, every attempt is made to keep such variables at a minimum, but with conventional equipment and under conventional procedures, these effects result in a greater variability of excitation and hence greater analytical errors than would be predicted by simple reproducibility tests. Obviously, reproducibility is of little value if it is found that the result reproducibly obtained on a particular sample

is in error because of some unknown difference between the sample analyzed and the standard sample.

Aluminum and magnesium alloys are particularly prone to show changes in the relative excitation of different wave lengths with changes in microstructure or metallurgical history. In routine analysis it is highly desirable, therefore, that the dimensions and form of the electrodes and the metallurgical history be carefully reproduced. When differences of this sort necessarily exist between analysis samples and standard samples, the quantitative effect of the differences must be evaluated and a correction applied in calculating the composition of the sample.

In routine analysis of aluminum alloys, it has been found that with spark excitation, the average deviation between spectrographic and chemical results usually lies between 2 and 3% of the amount present. Well over 90% of the results will show deviations of less than 5% and few, if any, will show deviations in excess of 10%. These figures are, of course, only approximations, since there are slight differences in the accuracy obtained on different elements and in different concentration ranges. The figures given are based on actual experience with relatively untrained spectrographic operators in routine laboratories and are less favorable but more pertinent than data taken in special tests undertaken to determine the best possible accuracy obtainable.

There are two main systems of applying the alternating current spark to the direct analysis of metal samples. Many laboratories, particularly in the steel industry, use two rod-shaped specimens as self-electrodes. Probably a greater number of laboratories use the system developed by Aluminum Research Laboratories involving the use of a machined flat surface of the sample as one electrode and a graphite rod as a counterelectrode. The choice between these two methods is often only a matter of convenience and usually depends on the forms in which samples are received, and on the standard samples available. Figure 2 shows the Petrey spark stand developed especially to handle disk-type samples.

The top plate of the spark stand serves both as support and electrical connection to the analytical sample. The lower electrode is high-purity graphite rod, 0.25 inch in diameter with a tapered end culminating in a hemisphere of about 0.06-inch radius. A freshly cut electrode is used for every test, and the gap is adjusted with the aid of the pivoting gap gage attached to the top plate on the left-hand side. The sample shown on the spark stand is a routine sample of the type regularly used by Aluminum Company of America, and consists of a circular disk originally 0.25 inch thick and 2.5 inches in diameter, cast in a cold steel mold. The sample as cast is usually recessed to a depth of about 0.10 inch in the central portion of one side to facilitate machining. The sample is so positioned on the spark stand that the center of the



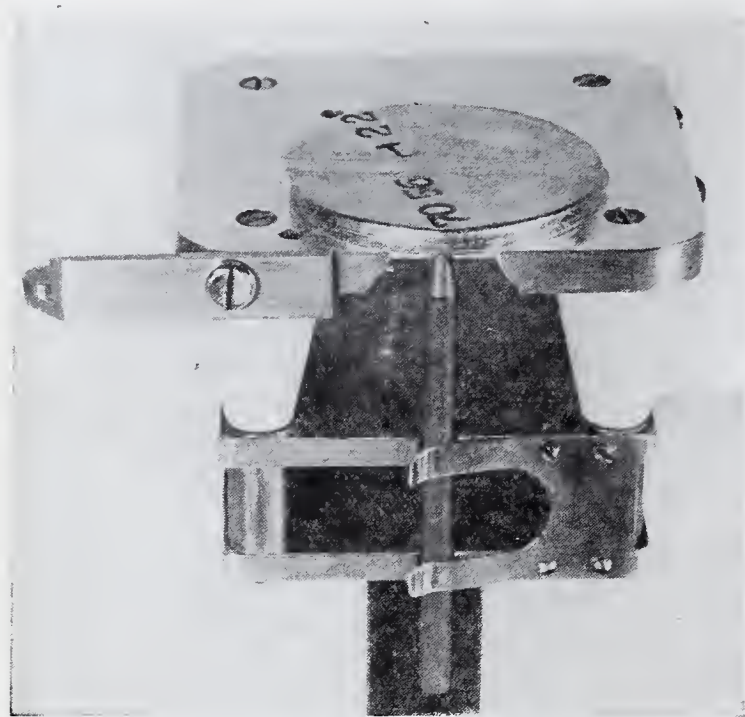


Figure 2. Petrey Spark Stand

spark falls approximately 0.375 inch from the edge of the sample and removed from the pouring sprue by an arc of  $90^\circ$  to  $150^\circ$ . The surface to be sparked is machined to a depth of 0.05 to 0.06 inch.

Following are the conditions of excitation employed on A.R.L.-Dietert controlled spark units in the routine laboratories of Aluminum Company of America:

Nominal power setting	2 kw.
Capacitance	0.021 mfd.
Self-inductance	1.44 mh.
Secondary resistance	None
Rated peak voltage of transformer	35,000
Primary current	6.6 amperes
Primary voltage	75 volts
Exposure	10 seconds
Prespark	10 seconds

The nominal power setting of 2 kw. on this unit actually represents a power input of about 1.3 kw. This represents a relatively high-powered spark, permitting the use of the rather short exposure time of 10 seconds. The self-inductance of 1.44 mh. is provided by employing the full self-inductance provided in the spark unit itself (0.36 mh.) plus three additional coils of 0.36 mh. each. The figure of 1.44 mh. is the nominal figure supplied by the manufacturer. The actual value has not been accurately measured in these laboratories and is not considered very important, for the reason that a large excess of self-inductance is used and the character of the discharge is not affected by small changes in self-inductance under the particular electrical conditions employed. There are three main reasons for using this unusually large amount of self-inductance: (1) It favors the excitation of certain arc lines required in the analysis of aluminum alloys. (2) It produces spectra of low spectral background and low in air lines. (3) When self-inductance is sufficiently high, accidental changes in self-inductance resulting from changes in relative positions of source unit, spectrograph, electrical leads, and miscellaneous metal objects in the vicinity of the spark unit have a negligible effect on the discharge.

This spark unit contains an inductive reactance in the primary which is "tuned" with respect to the capacitance and other circuit constants. In actual practice, this "tuning" is accomplished empirically by adjusting the air gap or number of turns in the reactance to produce a certain primary current for each of the three power settings of the instrument. On the 2-kw. power setting, the primary reactance is adjusted so that there will be a current of 6.6 amperes in the transformer primary when the voltage drop

across the primary is 75 volts. This primary voltage drop is controlled by adjusting the phase relationship between the synchronous gap and the secondary circuit by rotating the stator points of the synchronous gap. These somewhat arbitrary adjustments result in a spark which fires several milliseconds before the condensers reach maximum charge. When all the circuit constants are adjusted to the values given, a spark discharge is produced which compares favorably in stability, reproducibility, and sensitivity with that obtained on other conventional spark units in the analysis of aluminum alloys.

The selection of exposure times and prespark times in spark analysis should be based on the following considerations:

The intensity-time relationship of each spectrum line to be used.  
The change in stability of the spark with time.  
The effective photographic speed of the spectrograph.  
Considerations of speed and efficiency in operating the laboratory.

During the course of sparking a sample, the intensities of the various spectrum lines produced are usually changing more or less continuously. The rise in temperature of the electrodes, the accumulation of vapors in the spark, the oxidation of the electrodes, the depletion of the more volatile constituents in the area sparked, and the change in spark gap as the electrode tips are volatilized, all tend to cause changes in intensity during the spark exposure. Because of differences in physical and chemical properties, no two elements behave in precisely the same way and at any particular time, lines of two different elements may be changing in opposite directions. Difference in behavior between the elements is usually greatest and the reproducibility of intensity ratios poorest during the first few seconds of sparking. As the discharge proceeds, the rate of change of both individual line intensities and intensity ratios between different lines gradually diminishes as the various factors controlling intensities approach a quasi-equilibrium. If the sparking duration is prolonged sufficiently the reproducibility of intensity and intensity ratios again begins to deteriorate as the electrodes become pitted and encrusted with oxide. In most cases this condition obtains before the intensity ratios reach an equilibrium value. This is particularly true of aluminum and magnesium alloys. The 10-second prespark and 10-second exposure used in Aluminum Company of America laboratories are based on careful time *vs.* intensity studies, on statistical studies of reproducibility, and on considerations of time consumption and efficiency. As in the case of most other decisions in selecting optimum operating conditions, this decision was necessarily a compromise.

While the Petrey stand was originally designed to accommodate relatively massive samples on which a sufficiently large flat surface could be machined, many laboratories using this stand are called upon to handle other types of samples, such as wire, rods, rivets, drillings, powdered materials, and miscellaneous small fragments of materials. The Petrey stand can be conveniently adapted to the handling of metal samples of various sizes and shapes by the use of suitable clamps and holders. Powdered samples are usually formed into pellets by means of a briquetting press, with an addition of de-ashed natural graphite if the sample is a nonconductor of electricity or has poor briquetting properties. Such pellets are supported on the Petrey stand by special adapters and are sparked by essentially the technique used on metal electrodes.

RECENT DEVELOPMENTS IN EXCITATION UNITS. Recognizing the need for excitation units of higher reproducibility, greater sensitivity of detection, and greater freedom from metallurgical effects, a number of laboratories have attempted to improve on the conventional controlled spark. These efforts have proceeded along two main channels. One group of workers has concentrated its efforts along the lines of improving the controlled spark by close attention to the mechanical construction of the interrupter, the use of voltage regulators of various types, and the use of trans-



mers of higher voltage and higher power rating than those formerly used. That such measures are effective in improving the precision of analysis has been amply demonstrated in the steel and motor car industries. Other workers have concentrated on more fundamental changes in the design of the excitation unit, directing their efforts towards eliminating some of the defects and limitations inherent in the conventional controlled spark.

One such unit, described by Hasler and Dietert (5) and Hasler and Kemp (7), has been used in several laboratories as a replacement for the direct current arc, alternating current arc, and alternating current spark in the analysis of both metallic and non-metallic samples. This unit is a flexible apparatus, producing a variety of discharges similar to the conventional alternating current and direct current arcs and sparks produced by other excitation units, as well as discharges of an intermediate nature not falling within the usual meaning of the terms, alternating current arc, alternating current spark, or direct current arc.

The apparatus consists essentially of a power circuit powered by a 1000-volt, 5-kw. transformer and an initiator circuit consisting of a low-powered, high-voltage transformer, a condenser, and a synchronous interrupter. The power circuit includes an inductance, a capacitance, and a resistance, all three being variable over a rather wide range. Power circuit and initiator circuit are connected across the analytical gap in parallel and are rectified in the same sense. Because of the low voltage of the power circuit, the use of much larger capacitances than those employed in ordinary spark units is feasible, and resistance becomes an important and useful variable in controlling the nature of the discharge. The relatively low-powered initiator contributes only slightly to the spectra produced and, therefore, may be regarded only as a timer. The removal of the synchronous interrupter from the discharge circuit is an important improvement over the controlled spark unit in which a part of the intensity variations is traceable to the interrupter. Other features include a built-in voltage regulator actuated by the discharge circuit, a built-in microphotograph connected to show either the charge or discharge plates, or both, a peak voltmeter in the discharge circuit, ultraviolet irradiation of the synchronous gap, "wiper contacts" on the shaft of the interrupter motor arranged so as to short the charge condensers after each discharge train, and convenient controls for varying any of the circuit constants independently.

This instrument is too new to be judged on a basis of practical routine performance, but sufficient evidence has already accumulated to indicate that this unit, or units of similar fundamental design, may produce substantial improvements in certain routine analyses. No really comprehensive investigation of this source has yet been made, and because of the large number of combinations and permutations of electrical conditions provided, it will probably be many months before optimum conditions are definitely established for even the most common analyses. At Aluminum Research Laboratories, two types of discharge produced by this type of unit have been found extremely useful, one which will give spectra of excellent reproducibility when applied to most elements generally determined by spark technique, and one which will give remarkably good sensitivity of detection. Following are the circuit constants and general characteristics of these two discharges:

	Type A	Type B
Voltage	940 volts	940 volts
Capacitance	5 mfd.	60 mfd.
Inductance	480 microns	480 microns
Resistance	100 ohms	50 ohms
Sensitivity	Comparable to A.C. spark	Equal to or better than D.C. arc
Reproducibility	Comparable to A.C. spark	Better than A.C. arc
Accuracy	Better than A.C. spark	Better than A.C. arc

Type A produces results having approximately the same reproducibility as the controlled spark but yielding somewhat better accuracy in some cases because of a marked reduction in effects caused by the presence of other elements and by the metallurgical history of the sample. The Type B discharge appears to be superior in almost every respect to any discharge of comparable sensitivity of detection which can be produced on conventional arc or spark equipment.

While the results obtained under the conditions described indicate that such an excitation source is more generally useful than any other unit commercially available at the present time, it is

very unlikely that these conditions are the optimum for the analysis of metals other than aluminum, since no comprehensive investigation has yet been made along these lines.

While virtually all the routine metallurgical analysis of Aluminum Company of America laboratories is now carried out on controlled spark units, it is probable that the spark units will be greatly modified or replaced by excitation units now under development. In the remainder of this paper, only the controlled spark units of the type currently used are considered.

## THE SPECTROGRAPH

In the selection of spectrographic equipment, the spectrograph itself usually represents the least difficult problem of any of the main pieces of equipment. Except for certain specialized work, any of the popular spectrographs are adequate for most routine applications. Very often the selection of a spectrograph is guided more by availability, policy, and personal preference than by any important difference in optics or mechanical design. The following considerations, however, should not be overlooked in the selection of the spectrograph:

Resolving power.

Linear dispersion (Ångströms per mm. at the plate or film, not angular dispersion as expressed by the physicist).

Wave-length range covered by a single spectrogram.

Relative amount of scattered light ("Littrow fog", for example).

Photographic speed.

Quality of the slit.

Flexibility.

With respect to resolving power and linear dispersion, any of the larger standard prism spectrographs or the available grating instruments using original gratings are adequate. While there has been some prejudice against 1.5-meter grating instruments in the ferrous field, recent applications in industry seem to indicate the adequacy of these instruments for steel analysis. Actually, the dispersion of a spectrograph is less important than resolving power in any comparison among popular makes. Present-day spectroscopic plates and films have sufficiently fine-grained emulsions to shift the emphasis from dispersion to resolving power. In a number of instances where closely adjacent lines must be used in the analysis of various materials at Aluminum Research Laboratories, it has been found that a 1.5-meter grating instrument yields slightly better results than the standard prism instruments at wave lengths above about 2800 Ångströms. Part of this effect appears to be caused by the inherent difference in resolving power and part by the fact that the particular grating instrument used shows less deviation from theoretical performance under conditions of changing temperature, mechanical shock, and hard usage. For very complicated spectra, such as those obtained from rare earth elements or elements of very high atomic number, any of the prism instruments or small fixed-setting grating instruments may be inadequate. For such work, larger more flexible grating instruments are usually required.

The wave-length range which can be photographed in a single spectrogram is very important in some applications. The advantages of being able to include in a single spectrogram suitable lines of all the elements to be determined in a sample are obvious. It is even more important in qualitative analysis, where occasionally the spectrographer is called upon for a complete qualitative analysis on a sample too small for more than one test. Greater dispersion, therefore, may be a disadvantage in some cases and it is usually necessary to compromise between dispersion and wave-length range. Among prism instruments of relatively high dispersion, an available Wadsworth-type spectrograph has the advantage of the additional spectrum range afforded by use of a 14-inch plate in lieu of the 10-inch plate used on comparable prism instruments. This is carried further on one of the newer grating spectrographs which uses either two 10-inch plates placed end to end or a single 20-inch film or plate.



Table I. Characteristics of Several Makes of Spectrographs

	A.R.L.- Dietert	Baird (Large)	B. & L. (Littrow)	Gaertner (2 Lens)	Hilger (Littrow)	Jarrell- Ash (Grating)
Resolving power <sup>a</sup>	2	1	3	3	3	1
Dispersion <sup>a</sup>	3	1	2	2	2	1
Wave-length range (single spectro- gram)	2	4	3	1	3	2
Scattered light	2	1	2	1	2	1
Resistance to vibra- tion and shock	1	1	2	2	1	2
Auxiliary equipment available	1	2	3	3	2	2
Photographic speed	2	3	1	1	1	.. <sup>b</sup>
Slit	Adjust- able	Fixed	Adjust- able	Adjust- able	Adjust- able	.. <sup>b</sup>
Wave-length setting	Semi-fixed	Continu- ously variable	Stepwise variable	Continu- ously variable	Continu- ously variable	Continu- ously variable

<sup>a</sup> Comparison limited to wave lengths most widely used in metallurgical analysis (2000–4000 Å.).

<sup>b</sup> No data available as yet.

NOTE. The numbers, 1, 2, and 3 denote the rank of the various instruments when arranged in descending order with respect to the particular attribute or quality compared, based on the manufacturers' literature and the author's experience.

In the determination of minor impurities with spectral sources of very high total intensities and when long exposures are required, a troublesome effect is encountered on all Littrow spectrographs. This effect, often referred to as Littrow fog, is a general fogging of the plate or film by the light reflected from the front face of the collimator lens. This inherent defect in the Littrow design is ameliorated to some degree in most Littrow spectrographs by masking the center of the lens or by tilting the lens slightly. The effect is eliminated in the Wadsworth-type prism spectrograph and scattered light of this sort is not encountered to any large degree on most grating spectrographs.

Most of the popular commercial spectrographs have similar photographic speeds. Grating spectrographs are more variable in this respect because of the poor reproducibility of the reflectivity of gratings. Grating instruments also differ appreciably as to relative response at different wave lengths, even among spectrographs of the same make. The relative speed of the grating at different wave lengths can be roughly controlled by the method of ruling. A grating ruled in such a way as to distribute the light intensity as evenly as possible among orders or wave lengths is usually a relatively slow grating at any specific wave length. When two or more grating instruments are to be used interchangeably in the same laboratory, it is desirable that the gratings be approximately matched by selection.

Most of the commercial spectrographs have slits of adjustable width. In the opinion of the author, most of the adjustable slits supplied on the popular makes of spectrographs are unsatisfactory. The laboratories of Aluminum Company of America have encountered a considerable amount of trouble and annoyance from inaccurate graduations, poor reproducibility of slit width, nonparallelism of jaws, damage by untrained operators, and sticking of the adjustment mechanism. Most of these defective slits have been replaced by commercially available fixed slits.

An important characteristic of a spectrograph is its flexibility—that is, the ease with which its wave-length range may be changed and the convenience and speed of adapting the apparatus successively to widely different problems. The latter depends to a larger extent on the optical bench and accessories than on the spectrograph itself. Most of the larger prism and grating spectrographs are of the so-called automatic type, an automatic spectrograph being one whose wave-length range may be adjusted by means of a single control, either a simple crank or a switch governing a motor. Among prism spectrographs, those which have a single control which simultaneously alters prism rotation, distance from collimator to plate or slit, and plate tilt in nonstepwise fashion are more satisfactory than those on which a number of fixed wave-length settings are provided.

Several of the large grating spectrographs have satisfactory motor-driven mechanisms for changing wave-length range. The 1.5-meter grating instruments used widely in nonferrous analysis may be termed semiadjustable instruments. Camera and grating are permanently located on a diameter of the Rowland circle, and the wave-length range is determined by the position of the optical bench. The instrument is usually so arranged that the bench can be placed at two or three different positions to produce two or three wave-length ranges. When more than one wave-length region is required at frequent intervals, it is impractical to move the bench and it is customary to provide a permanent bench for each wave-length range required.

Whether or not it is important or even desirable to have a spectrograph whose wave-length range can be changed conveniently depends, of course, on the nature of the analytical work to be handled.

Research-type laboratories or any laboratories handling a wide variety of materials require flexible instruments. In some routine applications where a single wave-length range suffices for all analyses, as in the case of some routine aluminum laboratories, a fixed setting instrument may be preferable.

Table I summarizes a comparison among spectrographs used in metallurgical analysis in the United States. The so-called "medium" quartz spectrographs have been omitted because of the fact that these instruments are not used in modern industrial applications to as large an extent as the larger instruments. It should not be inferred, however, that the medium quartz instrument is necessarily inferior in all applications. These smaller instruments are adequate for many analyses of materials yielding relatively simple spectra, and in some work, the much greater wave-length range covered by a single spectrogram may be an important advantage. Nonferrous metal laboratories in Canada, Great Britain, and Germany have made rather wide use of medium quartz instruments. Excellent medium quartz spectrographs have been produced by a number of manufacturers. Today, in the United States, most spectrographic laboratories occasionally encounter analytical problems which require instruments of higher dispersion. Accordingly, since the cost of the spectrograph is small compared to the saving in analytical costs in replacing wet chemical methods with spectrographic methods, most industrial laboratories prefer to use one of the larger prism or grating instruments.

In general, the mode of use and the optical accessories are essentially the same for the various types of spectrograph. On any spectrograph, the external optical system is as important as the optical system in the spectrograph itself in making quantitative analyses. There are three main considerations in selecting the external system to be used on any spectrograph: (1) A representative or reproducible sample of the emitted light must be presented to the spectrograph. (2) The exposed portion of the spectrographic slit (or secondary aperture, in the case of astigmatic instruments) must be uniformly illuminated. (3) The light entering the collimator or grating must equally represent all points on the slit or secondary aperture. Also to be considered in the selection of the optical system is the effective photographic speed of the instrument.

The most generally satisfactory optical system consists of a lens placed near the slit or secondary aperture of the spectrograph, with the analysis gap so positioned with respect to the lens that an image of the gap is focused on the grating or collimator. The focal length of the lens employed depends upon what portion of the spark is to be sampled and on the photographic speed required. The maximum photographic speed which can be obtained without impairing the spectral line uniformity is produced by using a focal length such that the source



age precisely fills the collimator or grating. In spark work, the entire spark column, or a carefully limited central section of the spark, is usually sampled. In most laboratories spherical lenses are used for this purpose. However, some laboratories prefer to use a cylindrical lens placed with its axis horizontal, so that focus is obtained only in the vertical direction. Either a cylindrical or spherical lens used in the above manner will produce spectrum lines uniform along their lengths and will satisfy the requirements of light sampling. The spherical lens yields higher photographic speeds on astigmatic spectrographs, while the cylindrical lens is somewhat less critical in its positioning from side to side and makes the wandering of the spark or arc around the electrodes less effective in changing the intensity reaching the grating or prism. For many purposes, no condensing lens is required. In this case, there is a certain minimum distance from source to spectrograph, below which the previously mentioned requirements of the optical system are not met.

Spectrographic laboratories of Aluminum Company of America employ all the general types of spectrographs discussed. In routine work these instruments are used interchangeably by essentially the same techniques and with only those modifications and accessories necessary to suit the physical dimensions of the instruments and to compensate for differences in focal length and photographic speed.

#### PHOTOGRAPHY OF THE SPECTRUM

The next problem in the development of a spectrographic method is the photography of the spectrum. While it is probable that direct quantitative interpretation of the spectrum by photoelectric means will eventually supersede photography to a great extent, it is even more probable that photography will remain one of the main problems of the spectrographic analyst for some time to come. Direct-reading instruments, such as the photometer (6), have been described in the literature, but such apparatus is not as yet commercially available. Accordingly, this discussion will be limited to photographic techniques of recording and measuring spectrum lines.

Among spectrographers there exists a diversity of opinion as to the relative merits of glass plates and films in quantitative work. The consensus of opinion seems to be that film is more consistent in contrast and response than plates. On the other hand, film is more difficult to handle during processing and densitometry, and only one available densitometer is equipped with a film holder. In Aluminum Company of America laboratories, considerable annoyance has arisen because of defective films and plates, the occurrence of defects being more frequent in plates than in film. Usually the choice between film and plates has been made by the manufacturer of the spectrograph, and most commercial instruments are equipped to handle one or the other, but not both. Similar emulsion types are available for both films and plates and the following comments apply to both:

In selecting a photographic emulsion for a particular analytical job, the most important considerations are response of the film to the wave lengths required, contrast and change in contrast with wave length, grain size of the emulsion, and ease and speed of processing. The following emulsions have been selected on the basis of the foregoing considerations for use in Aluminum Company of America laboratories:

Emulsion	Application
Spectrum Analysis No. 1	Routine analysis of aluminum and magnesium
3-F, W. & W. process panchromatic	General qualitative and quantitative tests for all elements except potassium
L	Qualitative and quantitative analysis, including potassium
N	Specific tests for potassium

Spectrum Analysis No. 1 (probably the most widely used spectroscopic emulsion in the United States) is fine grained, has high contrast (gamma—1.9 to 2.0 as used in Aluminum Company

of America laboratories), satisfactory speed, and excellent processing characteristics. Its principal defect is its relatively rapid change of gamma with wave length. Ideally, of course, the emulsion should have the same gamma at any two wave lengths to be used in combination with each other. Although emulsions superior to Spectrum Analysis No. 1 in this respect have been developed, they have been objectionably slow in processing, somewhat coarse grained, and, in the case of film, inconvenient to handle on a routine basis because of excessive curling. It is the hope of most spectrographers that a film of uniform gamma, having the other desirable characteristics of Spectrum Analysis No. 1, will become available. Within rather broad limits the actual value of gamma is less important than the variation of gamma with wave length. A high gamma, if reproducible, makes densitometric measurements less critical, while a low gamma permits the use of a particular set of wave lengths over a larger range of concentrations.

In quantitative spectrographic analysis the development and processing of the photographic emulsion are highly important. Composition, agitation, and temperature of the developer must be reproduced very closely. The effective strength of a freshly prepared batch of developer changes rather rapidly on exposure to air and by depletion during the first few films or plates developed in it, but the change becomes more gradual thereafter. The use of fresh developer for every film or plate is not satisfactory because of variation in the amount of oxidation occurring during preparation and storage, and because of differences produced by minor variations in technique and purity of chemicals used. A satisfactory means of maintaining developer at sufficiently constant activity is the periodic replacement of a part of the developer in the tray with new developer. In Aluminum Company of America routine laboratories, half of the developer in the tray is replaced with new developer at regular intervals ranging from 2 to 4 hours, depending on the quantities of film or plates developed.

The temperature of the developer and the rate and method of agitation are also highly important. The agitation should be accomplished in such a way that the same amount of movement of the developer is produced at all points on the emulsion. No commercially available spectrographic developing machines are altogether adequate in this respect. Developing machines using longitudinally rocking trays produce slightly greater development near the ends of the trays and must be carefully regulated to prevent standing waves in the developing solution. Brushing the emulsion during development is a recognized technique for high-quality photographic work, but the brushing action must be accomplished mechanically rather than manually to obtain the desired reproducibility of development. Agitation by paddles moving close to, but not touching, the film has been suggested, and a wide variety of developing machines providing various types of oscillatory movement of the plate or developing solution, or both, have been used successfully.

One developing machine easily adaptable to routine spectrographic work is available commercially. While this machine provides only a simple longitudinal rocking motion, thereby falling short of the ideal machine for the most uniform development, it does have an acceptable thermostatic temperature-control system and has given reasonably satisfactory service in many routine laboratories. Machines of this type used in laboratories of Aluminum Company of America have given satisfactory results when properly adjusted. In using such a machine it is necessary to maintain the volume of developer within rather close limits and to adjust the agitation rate accordingly. The films or plates will still show some nonuniformity in development along their lengths, but as long as the films or plates are always placed in the same position relative to the tray, the effects are so slight and so reproducible as largely to cancel out in the spectrographic calculations. It is hoped that improved developing machines will become available in the near future.



In most spectrographic laboratories, developer, shortstop, and fixing solutions are all contained in the developing machine. Combination shortstop and hardening solutions, and combination fixing and hardening solutions are sometimes used. One satisfactory combination, probably the most widely used in American routine laboratories, consists of a dilute acetic acid solution used as a shortstop, and a fixing solution supplied commercially in liquid form for x-ray work.

Following are the essential details of the development and processing of the technique used in the routine laboratories of Aluminum Company of America:

Development for 3 minutes in Eastman Formula D-19 at 65° F.

Immersion for 2 to 3 seconds in 5% solution of acetic acid.

Fixation in x-ray fixing solution. The film or plate is allowed to remain in the fixer a few seconds after clearing, the total immersion time being on the order of 10 to 15 seconds.

The emulsion is lightly swabbed with a dripping wet cellulose sponge and the excess water removed by wiping with an almost dry sponge.

Films are dried in an infrared film dryer. Plates are dried in a warm current of air, either in a commercially available machine or in an apparatus prepared locally. SA-No. 1 films or plates are dried in about 1.5 minutes. Most other emulsions require longer periods.

The above technique was not designed for maximum speed. The development time can be greatly reduced by using a stronger developer or higher intensities, but there is some possibility of impairing reproducibility of development. Much more rapid developing and processing techniques are used successfully in laboratories in which high-speed analysis is a prime requisite, such as those of the Ford Motor Company and General Motors Corp.

Spectrographic emulsions are affected rather strongly by changes in relative humidity. For the most accurate work, it is highly desirable that the spectrographic laboratory be maintained at constant temperature and humidity.

#### PHOTOMETRY

With the exception of the emission source, the microphotometer is the most critical piece of equipment in the spectrographic laboratory. (Certain manufacturers apply the term "densitometer" to instruments of this type. In this paper the more accurately descriptive term "microphotometer" is applied to all makes.) Among the commercially available microphotometers, no one instrument will necessarily satisfy all the requirements of a particular laboratory. All available instruments have desirable and undesirable features and many laboratories have found it necessary either to build their own microphotometers or to modify purchased instruments. All the present standard makes of microphotometers have been improved and the most active manufacturers in the field plan substantial improvements when conditions permit. At present the choice among standard makes is largely governed by the photographic material to be used—i.e., whether film or plates—by the availability of the various makes, and by the preference for recording or nonrecording instruments. If more than one make is available for a particular application, the following qualities should be compared:

##### Stability.

Legibility of scale or chart paper and the precision with which it can be read.

Area of the emulsion whose transmission or density is being measured at any one instant.

Area and magnification of the field of view on the spectrogram.

Whether or not the instrument is equipped or can easily be equipped with a wave-length scale or comparison spectrogram.

Operator comfort and operator fatigue.

Stability is highly important, for if the instrument will not yield dependable, reproducible readings, the other considerations are meaningless. The galvanometer scale, meter scale, or whatever means are provided for registering the measured function of

density, should be sufficiently large for easy reading, should have sufficient number of graduations to yield readings of the desired precision with a minimum of interpolation, and should occupy such a position that the operator is not required to turn or incline his head in looking back and forth from spectrogram meter or galvanometer scale.

The area of the emulsion included by the beam of light reaching the photocell should be large enough to include a large number of emulsion grains, thus providing insurance against error caused by nonuniformity within the grains themselves and random differences between individual grains. Optimum slit dimensions are governed by the grain size of the emulsion, the width and transverse contour of the line images to be measured, and the available length of line on the film or plate. With modern fine-grained emulsions, it is generally conceded that 10,000 square microns is a satisfactory cross-sectional area for the light beam. The width of the beam should be substantially less than the width of the spectrum line to ensure that the microphotometer is permitted to yield a deflection representing the maximum blackness of the image, and the beam should be shorter than the width of the spectrum line by an amount sufficient to provide a practical margin of safety in adjusting the position of the spectrogram with respect to the beam. The beam should be provided with a rotary adjustment, so that it can be made parallel to the spectrum line image. If the light beam is rectangular, its maximum length is limited by the curvature of the spectrum line image in the case of prism instruments, and if it is desired that relative intensities of long spectrum lines be measured on prism spectrographs, a curved photometer beam is desirable. In analytical procedures designed for maximum speed on individual samples, the area covered by the light beam can be made relatively large by using long slits on the spectrograph. In procedures designed primarily for the most economical handling of large numbers of samples, as in the procedures used by Aluminum Company of America, relatively short slits are used in order to record a larger number of spectra per plate or film.

The area and magnification of the portion of the spectrogram visible to the operator during or immediately before measurement are important. The operator should be able to see a sufficient spectral range to enable him to orient himself quickly through the recognition of familiar groups of lines. The spectrogram should be magnified sufficiently to prevent the measurement of the wrong line when the desired line lies close to other lines and to permit the detection of dust particles or flaws in the emulsion or emulsion base which would introduce errors in measurement. In most applications, a magnification at least tenfold is desirable. For most purposes a field of view including 50 Ångströms is adequate if a satisfactory wave-length scale is used. If no wave-length scale is available a field of view of 100 or 200 Ångströms is usually desirable.

A wave-length scale or comparison spectrum, preferably both semipermanently mounted in the microphotometer, is a desirable accessory for operational speed and convenience. This is particularly true in the training of inexperienced operators in routine applications, where the operator must develop speed in measurement before becoming thoroughly familiar with the spectra involved in the analysis. As operators become more experienced the wave-length scale and standard spectrum are used less and less until finally they are used only for occasional reference.

Considerations of operator comfort and fatigue should be given a great deal of weight in the selection of an instrument for routine use. Several otherwise excellent microphotometers have been faulty in this respect and an instrument which is quite satisfactory for occasional use in research type work may be altogether inadequate for continuous use in a routine application because of the failure of the designer to consider the comfort of the operator. The following points are important in this connection.

Adequate illumination of all meters, scales, and dials regularly read during measurement.



Magnification, illumination, and clarity of the spectrogram image viewed during measurement.

Positions of spectrogram image and scale registering the measurement. Both should be approximately at eye level and close together.

Arrangement and ease of manipulation of controls.

Posture of operator during measurement. Instruments which require the operator to work with head or back inclined are particularly undesirable in continuous operation. The most generally satisfactory instruments with respect to operator fatigue are those in which the operator sits with back and head erect and can view both the spectrogram and the galvanometer or meter dial at approximately eye level.

When two or more microphotometers are to be used interchangeably in the same laboratory, a further requirement must be met. The relationship between deflection and photographic density should be the same for all the instruments to be used interchangeably. Otherwise, a separate emulsion calibration would be required for each instrument, causing complication and possible confusion in the analytical operations. For this reason, the Aluminum Company of America laboratories, microphotometers which are very nearly linear or can be adjusted to linearity are preferred. By a linear microphotometer is meant one whose deflections are directly proportional to the transmission of the photographic emulsion.

The amount of scattered light reaching the photocell is a very important consideration in the design of a microphotometer. However, in the author's experience, scattered light has been of secondary importance in choosing between standard makes of microphotometers, simply because the available instruments have been reasonably satisfactory in this respect. The most important effects of scattered light are the loss of sensitivity in the measurement of weak lines and the change in the response curve (the curve relating deflection and transmission). Since the latter is automatically taken care of in calibration, an increase in the proportion of scattered light merely necessitates higher reproducibility of deflections and greater precision in reading deflections. All other considerations being equal, an instrument producing a minimum of scattered light obviously is desirable.

The arrangement and mechanism of the various controls on the microphotometer are important in any particular application, but the requirements vary considerably among different applications. In mass production work, where greater emphasis is placed on economy than on the speed of an individual analysis, large numbers of spectra of relatively small height are used. In such applications an accurate rack and pinion mechanism or its equivalent is required for rapidly adjusting the position of the spectrogram prior to measurement. In high-speed analysis using spectra of larger height, the vertical positioning of the spectra is as critical and a manual sliding adjustment may be preferred for mechanical simplicity. In all cases, the controls should be arranged for maximum accessibility and ease of manipulation. Elaborate and complicated controls, either electrical or mechanical, should be avoided.

In the accurate measurement of a spectrum line, the speed of scanning the line is limited by the period of the galvanometer, meter, or other registering mechanism. If the line is scanned too rapidly, the proper deflection obviously will not be attained. Equally obvious is the desirability of scanning as rapidly as possible for economy of time. Hence, there is an optimum scanning speed which depends on the characteristics of the particular microphotometer and the width of the spectrum lines. In order to ensure a close approximation of this optimum scanning speed, a motor-driven scanning mechanism is to be preferred, providing that the mechanism is not overcomplicated. However, at least one very satisfactory standard make employs manual scanning. Regardless of the means provided for scanning, at least two types of movement parallel to the spectrum should be provided—a coarse movement for positioning the spectrogram prior to measurement, and a fine motion for actual scanning. One excellent microphotometer actually provides four

Table II. Characteristics of Three Makes of Microphotometers

	Leeds & Northrup	University of Michigan	A.R.L.-Dietert
Stability	1	2	3
Legibility of scale	1	1	1
Densitometered area	3	1	2
Field of view	3	2	1
Linearity	2	2	1
Provision for wave-length scale	No	No	Yes
Provision for reference spectrogram	No	Yes	Yes
Scanning	Synchronous motor	Manual	Synchronous motor
Racking	Rack and pinion	Manual	Rack and pinion
Plate or film holder	Plate	Plate	Plate and film
Position of film or plate	Vertical	Horizontal	Horizontal
Necessity for optical alignment	Each plate	Occasional	Occasional
Amplifier	Yes	No	Yes

NOTE. Numbers 1, 2, and 3 denote rank of instruments when arranged in descending order with respect to the particular attribute or quality compared, based on manufacturers' literature and the author's experience.

such movements, the carriage being movable by manually pushing on it, by turning a crank, or by the use of a vernier knob, while the fourth motion is provided by the motor-driven movement of a receiver slit during scanning. At least one of these movements is superfluous.

Aside from the many successful instruments built by the users themselves or custom-built by instrument manufacturers, there are at least three satisfactory microphotometers available to American industry. Table II gives a comparison of these three instruments based on the experience of laboratories using them.

In laboratories employing spectrographs using 35-mm. film, the choice of the microphotometer is virtually limited to one designed specifically for the measurement of film. Most available instruments not only lack any provision for mounting film, but are very sensitive to the focus of the light beam on the emulsion or the focus of the spectrogram image on a slit. The difficulty of mounting a film in such a way that it will lie exactly in a plane perpendicular to the optic axis makes the application of several available microphotometers to the measurement of 35-mm. film rather impractical.

While a number of other makes of microphotometers are used in analytical work, only those which are currently available and are applicable to the methods described are included in this comparison.

Since most of the manipulative steps in photometric measurement are dictated by the design of the instrument used, there are only a few points in connection with the technique which require discussion. In most spectrographic procedures, the microphotometer is adjusted to yield certain standard deflections for unexposed emulsion and for complete opacity. On most conventional instruments, the adjustment is so made that a scale reading of 100 is obtained on unexposed emulsion and a reading of zero on an image of infinite density. If such an instrument is linear, the deflection registered represents per cent light transmission of the emulsion area measured. Unexposed emulsion is to be preferred to spectral background for adjusting the maximum deflection, even when significant amounts of spectral background are superimposed on the lines to be measured. In some laboratories, the deflection is set to the desired maximum in the spectrum itself at some selected point near the line to be measured. This is done as a means of correcting for spectral background and serves as such a correction in the proper direction, but not usually of the proper magnitude. This practice often results in greater errors in final analytical results than setting on unexposed emulsion and ignoring spectral background. The best procedure in most analyses is to do everything possible to keep spectral background to a minimum and then to correct for it either rigorously or not at all. Corrections for spectral background will be discussed further under "Calibrations and Calculations".



Two routine systems of photometric measurement are used in Aluminum Company of America laboratories. These are generally referred to as the vertical system and the horizontal system. In the vertical system, the operator measures a spectrum line of a given wave length in all spectra on the film or plate before proceeding to the next line. In the horizontal system, the operator measures the internal standard line and all the analysis lines in one spectrum before proceeding to the next spectrum. The vertical system requires somewhat less time for a group of samples but an instrument of greater stability.

The vertical system of measurement is used in those routine laboratories of Aluminum Company of America which are equipped with recording microphotometers. The high stability of the recording instruments used removes the principal disadvantage of vertical reading. Moreover, the arrangement of controls and the lack of any adequate comparator feature make horizontal reading impractical on the particular instruments used.

In Aluminum Company of America laboratories using non-recording instruments, the horizontal system is usually employed for three main reasons: (1) Calculations are made concurrently with densitometric measurements and calculation is faster and more convenient when all data on a particular sample are presented in sequence before proceeding to the next sample. (2) The excellent field of view and convenient controls of the particular instruments employed largely eliminate the disadvantages of horizontal reading on a less conveniently arranged instrument. (3) Horizontal reading is slightly more accurate, since the effects of any drift of the microphotometer are minimized.

In the laboratories using this system, the adjustment of zero and clear film deflection is made before starting to measure the first spectrum and is checked between spectra at intervals whose frequency depends upon the stability of the microphotometer. In no case, however, is the zero or clear film deflection deliberately changed or adjusted during or between measurements on the same spectrum. If the validity of the adjustment is in doubt during the course of measuring a spectrogram, the settings are checked and the entire set of lines remeasured for the spectrum. Differences in unexposed film transmission at different locations on the film are ignored and the clear film deflection is always adjusted at the same location on the film. Corrective adjustments, intended to reduce errors caused by differences in unexposed emulsion readings at different locations on the film, have been found actually to increase the errors in routine analysis because the uncertainty of setting is directly effective in introducing an error in the measurement of intensity ratios. When the lines are measured with the same setting of the instrument, even though that setting be subjected to appreciable error (as much as 1 or 2% in routine work), the effect of the uncertainty of adjustment on the analysis line is virtually cancelled by the effect on the internal standard.

In most laboratories, pairs of operators are used as microphotometer-calculator teams. One member of the team operates the microphotometer and reads the deflections aloud, and the other makes the calculations and copies the data, often making out the final report as fast as measurements are made. Teams operating in this way have attained speeds of well over 400 reported determinations per elapsed hour in laboratories organized for large mass production of analyses.

While the actual manipulations of measurement are simple and any reasonably dexterous person can attain the speed and dependability of measurement required, the microphotometer team in a routine laboratory must carry the largest part of the responsibility for the entire analytical procedure. Of all the crew members, they are best situated to observe both the end results, the symptoms of maladjusted apparatus, or faulty technique. They must detect faults or symptoms of faults in the procedure and apparatus, such as the following:

- Out of focus spectra.
- Misaligned spectrograph slit.
- Obstructions in the slit or elsewhere in the light path of the spectrograph.
- Poor line uniformity because of misaligned optics on the spectrograph.
- Poor reproducibility of absolute densities or of duplicate analyses.
- Excessive changes in emulsion or concentration calibrations.
- Excessively weak or strong spectrograms.
- Evidences of defective development or processing technique on the films or plates.
- Fogging of films by friction in camera exit.
- Fogging of films or plates by light leaks in spectrograph darkroom.
- Scratched, dirty, or originally defective films or plates.
- Erraticness of microphotometer when spark unit is turned on.
- This may be either the result of poor electrical shield or symptomatic of a fault in the spark unit.

In addition, the microphotometer team must select the analyses to be rechecked, must recognize unusual or unreasonable results, and must detect and report unexpected elements not included in the regular routine analysis. They must also detect any defects in the operation of the microphotometer, and should be able to make the necessary adjustments, replacements, or minor repairs necessary for efficient, uninterrupted operation.

## CALIBRATIONS AND CALCULATIONS

**GENERAL CONSIDERATIONS.** Methods for the quantitative interpretation of emission spectra fall into two general classes: comparison standard methods and internal standard methods. The term "comparison standard" is applied to all methods in which concentration is deduced from the blackness of the analysis line in the spectrum of the sample as compared to its blackness in spectra of standards. Internal standard methods embrace methods in which concentration is determined through its relationship to the intensity ratio of a line of the element to be determined and a line of another element present in constant amount.

Comparison standard methods are used, at least to a limited extent, in most general analytical laboratories. While, in general, they are less accurate than internal standard methods, they are often more practical or convenient, particularly in cases where an internal standard line of suitable characteristics is not available. In the determination of minor impurities, comparison standard methods are often adequate and in a few special cases the absolute reproducibility of intensities is such that the comparison standard method approaches the internal standard method in accuracy.

The internal standard method owes its general superiority to the fact that variations in the internal standard line provide automatic correction for variations in excitation, optical alignment, exposure, and, to a limited extent, for variations in the response of the photographic emulsion. In the internal standard method it is assumed that all variables other than concentration affect the measured intensities of the analysis line and internal standard line to the same degree. Unfortunately, this assumption is not rigorously correct. Except in cases where the ionization and excitation potentials of the two elements are similar, changes in electrical conditions may affect the two lines differently. Moreover, differences in boiling point, stability of compounds, or self-reversal effects may also affect intensity ratios. In addition to these factors affecting the actual intensity ratio, a number of factors may affect the measurement of the relative intensities of the two lines in such a way as to affect the apparent intensity ratios. Misalignment of the source with respect to the optics, or of the optics in relation to each other, may produce a substantial change in apparent intensity ratio in cases where the intensity distribution of the two wave lengths is different in the source. (In other words, the sampling of the two wave lengths may not be proportional.)



The internal standard system corrects for variations in exposure time only to the extent that the reciprocity law of the photographic plate holds. Variations in the response of the photographic emulsion introduce errors in intensity ratios unless taken care of by calibration corrections. In view of the effects of these variables which influence intensity ratios, it is obvious that while the internal standard method tends to correct for the variables other than concentration, the correction it actually applies may not be precisely accurate. Since the magnitude of these effects decreases as the reproducibility of absolute intensity increases, every effort should be made towards the elimination of all variables which will affect intensities or intensity measurements. Whenever possible, measurements are restricted to line pairs of very similar excitation characteristics. Of course, in many analyses the analyst has little choice in the selection of lines. In the analysis of aluminum alloys, for example, the choice of lines available is so limited that little attention can be paid to atomic origins. In all applications, the validity of the fundamental assumptions of the internal standard method and the reliability of the results obtained depend upon the reproducibility of excitation optics and photography. For maximum accuracy, the method resolves itself into one in which an attempt is made to remove all variables in absolute intensities and then an approximate correction is applied to take care of the residual variability of the overall procedure.

Bearing in mind the foregoing limitations, the basic assumption of the internal standard method can be expressed by the simple relationship

$$C = f(I_a/I_s)$$

where  $C$  is concentration,  $I_a$  and  $I_s$  are the relative intensities of the analysis line and internal standard line, respectively, and  $f$  is simply used as a symbol to denote a mathematical function of the single variable  $I_a/I_s$ . While no assumptions concerning the nature of this function need be made, the form of the analytical curves derived from experimental data indicates that the following equation holds within the range of concentration over which an internal standard line is used:

$$\log C = K_1 \log I_a/I_s + K_2$$

For most practical purposes  $K_1$  may be considered a constant, since the curves relating  $\log C$  and  $\log I_a/I_s$  generally have a negligible curvature in the relatively short range over which the particular line pair is used. Actually, of course,  $K_1$  is a variable which is a function of self-reversal and other excitation effects.  $K_2$  is virtually constant within any particular series of tests within a limited period of time, but has been found to vary over longer periods. Changes in  $K_2$  are simply manifestations of the familiar "curve-drift" which will be discussed later.

Somewhat more rigorous equations relating concentration and intensity ratio have been suggested, but they are of only academic interest to the spectrographer involved in practical, quantitative analysis. The only assumption of the internal standard method is to the relationship between concentrations and intensities that concentration is a function of the single variable,  $I_a/I_s$ .

**EMULSION CALIBRATION.** Since the internal standard method involves the determination of intensity ratios, the first step in the procedure is the conversion of the data obtained from the microphotometer to relative intensities. Until a few years ago, this was accomplished by simply restricting all photometric measurements to the approximately straight-line portion of the  $H_v$  and  $D_v$  curve of the plate and assuming that  $\log I = K \log T$ ,  $T$  being the photometric deflection. With this assumption, deflection ratios were used directly in place of intensity ratios. This method is still used by a few laboratories in this country and by a considerable number of British laboratories. However, the general consensus today favors the methods of somewhat wider scope and greater accuracy, involving the actual calibration of the photographic emulsion in terms of relative intensities. Fol-

lowing are the five principal methods of emulsion calibration used in quantitative spectrography:

1. *Inverse Square Method.* This method consists of simply preparing a number of exposures using the source at various measured distances from the spectrograph slit with no lenses or other optical parts intervening between source and slit. It is complicated to some degree by diffraction effects at the slit and by changes in the sampling of the light as the distance from source to slit is varied. On prism spectrographs the method has seen practical use in a number of applications. The accuracy obtained depends, of course, on the absolute reproducibility of intensity and exposure time. This fact, together with considerations of speed and convenience, makes the inverse square method impractical in most routine work and restricts its application largely to special research problems and to verification work supplementing other methods of calibration.

2. *Step Wedge or Step Filter Method.* A step wedge or step filter placed at the vertical focus of the spectrograph (at the slit of a stigmatic instrument, at the secondary focus of an astigmatic instrument) may be used for the simultaneous production of a number of exposures of known relative intensities. The relative transmission of the various steps with respect to the wave lengths at which the calibration is to be made must be known in advance or determined by some other means of calibration. The chief disadvantages of this method lie in its dependence on uniformity of illumination and in the unavailability of satisfactory filters or wedges with respect to quality and uniformity of relative transmission at different wave lengths.

3. *Step Sector Method.* This is the most widely used system of emulsion calibration in quantitative spectrography. The design and mechanism of the step sector and the method of preparing step spectrograms have been described repeatedly in the literature and in the instructions supplied by the manufacturers of the equipment.

In the step sector method a series of calibration spectrograms is produced simultaneously through the use of a rotating disk having a series of stepped apertures so arranged that the ratio of the exposures of any two successive steps will be a constant, usually in the neighborhood of 1.5 or 2.0. The sector disk is placed immediately in front of the slit on a stigmatic spectrograph and at the secondary focus of an astigmatic spectrograph. Most step sectors have from 4 to 9 steps, although only 2 steps are actually required. It is assumed that the rotation of the sector is sufficiently rapid that the relative exposures produced by the various apertures can be treated as relative intensities, and the calibration curve may be prepared by simply plotting microphotometer deflections as ordinates against relative exposures as abscissas for several spectrum lines and fitting the curves together by moving them laterally until they are partially superimposed.

A more accurate and convenient means for combining the data from a number of wave lengths or from different step spectrograms is provided by the Preliminary Curve Method. This method consists of first plotting each deflection against the deflection for the same wave length in the succeeding step of the step spectrogram. In other words, the deflection for any given intensity,  $I$ , is plotted against the deflection for an intensity of  $rI$ ,  $r$  being the ratio between the successive exposures. After all the data are plotted in this way on either linear or logarithmic coordinates, the best smooth curve is drawn and a series of data, which will represent the average of all the individual series, can be read from the curve and used to prepare the final emulsion calibration curve. The use of a preliminary curve is dealt with in greater detail in the Two-Line Method described below.

While its practical usefulness is evidenced by its wide use in industry, the step sector method has the following disadvantages:

- a. Large errors possible from stroboscopic effects with intermittent sources, such as the alternating current spark.
- b. Small errors introduced by effects of the intermittency introduced both by the sector and the source itself on the response of the photographic emulsion.
- c. Nonuniform illumination of the sector and nonuniform sampling of the light as a result of any defects in lenses or spectrograph slit, or as a result of target effects originating in nonuniform grating or prism.
- d. Highly critical adjustment of sector, light source, and lenses necessary for accurate work.
- e. Time consumption and inconvenience in having to make a special test under conditions usually differing from regular analytical exposure.

4. *Methods Involving Groups of Previously Calibrated Lines.* A calibration method involving the use of groups of iron lines whose relative intensities have been previously determined has been rather widely used for a number of years. In it, an attempt is made to select lines which not only cover the necessary range



of intensities in a single exposure but are invariant in relative intensity with respect to each other. The relative intensity values must be determined originally by one of the other calibration methods on the particular apparatus to be used in analysis. This method is particularly applicable to the analysis of iron and steel, since the iron spectrum affords a relatively wide selection of lines. The method, of course, can be applied to any other material by simply recording iron spectra on the plates or films for calibration purposes.

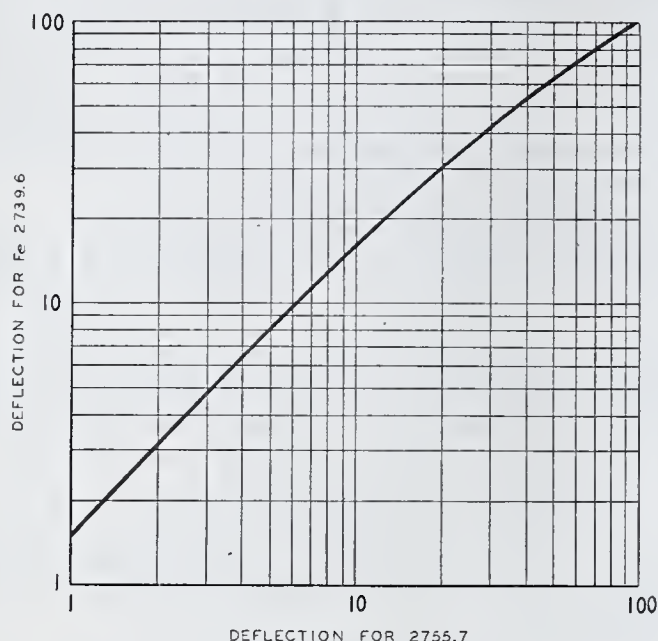


Figure 3. Preliminary Curve Used in Two-Line Calibration Method

In general, the method is superior to the step sector method and easier to use, once the large amount of preliminary experimental work has been done. As a matter of fact, the only serious objections are the relatively large amount of preliminary work which must be done in each laboratory and on each spectrograph and the direct dependence of analytical accuracy on the quality of this preliminary work. Attempts to transfer the relative intensity data on the calibration group from one type of apparatus to another have indicated that it is unsafe to base the work of one laboratory on a calibration group evaluated in another laboratory, unless the apparatus in the two laboratories is very similar and the excitation techniques are identical. The method could be based on calibration groups comprising lines of an element other than iron, but the author is unaware of any published works giving data on other elements. Most of the common non-ferrous alloys have spectra too poor in lines to be used directly for calibration purposes by this method. In such cases, the use of iron groups is feasible and simply requires the preparation of iron spectra whenever a calibration is to be made.

5. *Two-Line Method.* This method combines most of the advantages of the foregoing methods and avoids most of their disadvantages. Because it has not been published before, a more detailed treatment is given in the following section.

**TWO-LINE METHOD OF EMULSION CALIBRATION.** In the two-line method, as in foregoing calibration methods, the starting point in calibration is the accumulation of data consisting of a number of microphotometer readings on the photographed images of spectrum lines bearing some fixed ratio of intensity to each other. In all cases, one significant datum is provided by two readings, one representing an intensity weaker or stronger by some fixed ratio than the other. In the step sector method, this ratio is determined by the ratio between successive step sector apertures, while in the two-line system the ratio is fixed by the inherent intensity relationship between two spectrum lines of different wave lengths. The actual intensity ratio of these two lines need not be known except very roughly.

The line pair used in the two-line method must have the following qualities:

It must occur at accurately measurable densities in the routine spectrograms of at least a portion of the samples analyzed daily in the laboratory.

The intensity ratio between the two lines should lie somewhere between 1.2 and 2.0.

The intensity ratio between the two lines must remain constant under ordinary operating conditions and be independent of composition over the range of routine analysis in which the lines are to be used. This is accomplished by using two lines of the same element which have similar excitation characteristics.

The wave lengths of the two lines should differ by no more than 100 Å. (the less the better) and should lie more or less central with respect to the wave-length range over which the calibration is to be used.

The lines must be sharply defined and free from interference effects by other elements occurring in the alloy used for calibration.

In aluminum alloys, the iron spectrum affords the best opportunity for the selection of the calibration pair. Following are three line pairs which have been found satisfactory for use in routine laboratories engaged in the analysis of aluminum:

Line Pair	Intensity Ratio	Suitable Alloys
Fe (I) 3047.6/Fe (I) 3037.4	1.26	85, 122
Fe (I) 2966.9/Fe (I) 3037.4	1.56	85, 122
Fe (II) 2755.7/Fe (II) 2739.6	1.23	2S, 14S, 17S, 2S, 14S, 17S, 53S, 195

The intensity ratios given are based on determinations made under the following conditions:

Power setting	2 kw. <sup>a</sup>
Spark gap	3 mm.
Lower electrode	Graphite, 0.06-inch radius hemispherical tip
Upper electrodes	Machined aluminum disks
Spectrographs	Large two-lens prism, 1.5-meter grating

<sup>a</sup> Nominal value indicated by the switch markings on spark unit.

It has been found that these intensity ratios are accurately reproduced on grating and prism instruments in different laboratories under the above conditions, and are virtually unaffected by the variables present in the routine procedures used in Aluminum Company of America laboratories. The actual values of the intensity ratios given, of course, are subject to the systematic errors of the step spectrum method but, as was mentioned earlier, the precise value of the intensity ratio of the calibration pair need not be known in the two-line method.

To carry out an emulsion calibration by the two-line method, a series of spectrograms is prepared using a sample selected for routine production and containing iron in such quantity that the range of deflections over which emulsion calibration is desired can be produced with optics as the only variable. Exposure time, slit width, power, self-inductance, electrode arrangement, and other conditions of exposure and excitation, with the exception of the optical arrangement, should be the same as in routine analysis. The illumination should be varied from exposure to exposure in small steps. This may be done most conveniently on prism instruments by varying the distance from source to slit (using no condensing lens). On grating instruments the variation may be accomplished by changing the grating aperture or by using neutral screens or neutral filters. (The use of grating aperture as a variable with source image at grating requires that the line pair used shall have the same intensity ratio throughout the spectral range. For practical purposes, the lines given meet this requirement.) The series of exposures should include deflections over the entire range of deflections to be encountered in analysis. The films or plates must be processed under the same conditions as routine analytical spectrograms.

Both members of the line pair are measured in all the spectrograms, setting the full-scale deflection on an unexposed area of the film and measuring each member of the pair in each spectrum before proceeding to the next spectrum. For purposes of convenience, it will be assumed that the microphotometer is so designed and so adjusted that a reading of 100.0 is obtained on an unexposed emulsion and a reading of zero on an image of infinite density.

From the deflection data a graph is prepared, preferably on logarithmic coordinates, plotting each deflection recorded for one of the members of the calibration pair against the deflection for the other member in the same spectrum. The curve so obtained is called the preliminary curve. A typical preliminary curve for the line pair, Fe 2739.5/Fe 2755.7, shown in Figure 3, was prepared



red from data on Eastman SA No. 1 film. The sole purpose of which a curve is to provide a means of determining for any particular deflection produced by an intensity  $I$ , the deflection produced by an intensity equal to  $rI$ , where  $r$  is the intensity ratio of the calibration lines. This curve has the same function and significance as the preliminary curve in the step sector method, where it becomes the ratio between successive step sector apertures.

The final emulsion calibration curve is prepared from a series of points determined from the preliminary curve as follows:

As the initial point on the emulsion curve, select a deflection higher than any to be used in routine analysis, 98.0, for example. Refer this value to the preliminary curve and determine the deflection for  $r$  times the intensity represented by a deflection of 98.0. Referring to the example curve shown in Figure 3, where deflections for  $rI$  are plotted as abscissas, an ordinate of 98.0 corresponds to an abscissa of 96.7; 96.7 is now applied as ordinate and a third deflection is read on the abscissa scale. Proceed in this manner, recording each deflection value read from the ordinate scale and applying it as an abscissa to determine the next value, until deflections smaller than any used in routine analysis are obtained.

Table III shows the data so obtained from the preliminary curve in Figure 3. If the value of  $r$ , the intensity ratio of the calibration pair, is known, the data from Table I may be used to plot the familiar H. and D. curve. If the value of  $r$  is not precisely known, and for the most precise work it cannot be assumed to be known exactly, the photometric deflection,  $T$  or  $\log T$ , is plotted against  $\log a^n$ , where  $a$  is an arbitrarily selected number to be used in place of  $r$  and  $n$  is the exponent applied to  $r$  to obtain relative intensities (see column 1, Table III). The emulsion cali-

bration curves obtained when a value of 1.5 is assigned to  $a$  are shown by the solid lines in Figure 4. The numerical values for  $a^n$  are shown in the third column of Table III.

The calibration pair used to assemble these data was known to have an intensity ratio of 1.23. Had actual relative intensities been used as abscissas, the curve shown by the broken lines in Figure 4 would have been obtained. Either of these two curves could be used as the emulsion calibration curve, the only difference being that in the case represented by the broken curve, microphotometer deflections would be translated into relative intensities, while in the more general case represented by the solid line, all relative intensities would be raised to a constant power,  $\log a/\log r$ . Since, according to the basic assumptions of the internal standard method, concentration is a function of the single variable intensity ratio, it makes no difference whether or not numerical values of the relative intensities observed are raised to a power, as long as that power is a constant. It is, therefore, obvious that it is unnecessary to know the precise intensity of the calibration pair. (Similarly, in the step spectrum method it is unnecessary to know the ratio between the apertures, as long as the ratio between each two successive apertures is the same).

The emulsion calibration curve prepared in the two-line method is used in exactly the same way as the calibration curves prepared by the other methods described. The use of the arbitrary constant  $a$  in place of the intensity ratio of the calibration pair does not alter the mode of application of the calibration curve and does not affect any of the succeeding steps in the calculation procedure. For purposes of simplicity and clarity, the constant exponent introduced when  $a \neq r$  will be disregarded and it is understood that whenever relative intensity or intensity ratios are mentioned, the actual numerical values used may be raised to the power  $\log a/\log r$ . This will have no effect on the procedure or results.

**CALCULATION OF INTENSITY RATIOS.** The intensity ratio represented by the deflections produced by two spectrum lines may be determined by simply referring the deflections to the emulsion calibration curve and dividing the numerical values of relative intensity obtained. An easier and faster procedure is available when the emulsion calibration curve is mounted on a conventional calculating board with a sliding logarithmic abscissa scale.

For purposes of illustration, assume that the analysis line gives a deflection of 28.0 and the internal standard line a deflection of 11.6. Referring these deflections to the typical calibration curve (solid line) in Figure 5,  $I_a$ , the relative intensity for the analysis line, is found to be 0.152, and  $I_s$ , the relative intensity of the internal standard line, is 0.351. The intensity ratio then is  $0.152/0.351$  or 0.433. Since the numerical values assigned to the abscissa scale are only relative and since this scale is logarithmic, it is obvious that the value obtained for the intensity ratio is independent of the position of the scale relative to the curve. Accordingly, a more direct determination of  $I_a/I_s$  can be made by moving either the curve or the abscissa scale horizontally until  $I_s$  becomes unity, whereupon  $I_a/I_s$  becomes equal to  $I_a$ . In the specific example considered above, the curve would be moved to the position shown by the broken line, so that a deflection of 11.6 corresponded to a relative intensity of unity. The value of  $I_a/I_s$  is then the abscissa corresponding to a deflection of 28.0.

While the illustration used in the above example was for a log-log calibration curve as used on a nonrecording microphotometer, the same considerations apply and the same procedure is used on the semilogarithmic plot often used for recording microphotometers.

**PREPARATION OF EMULSION CALIBRATION SCALES.** When a nonrecording microphotometer is used in Aluminum Company of America laboratories the emulsion calibration curve is projected on a scale to be used slide rule fashion

Table III. Typical Data Used in Plotting Emulsion Calibration Curves

n	Deflection (Read from Preliminary Curve)	$a^n$	
		( $a = r = 1.23$ )	( $a = 1.50$ )
0	98.0	1.00	1.00
1	96.7	1.23	1.50
2	94.5	1.51	2.25
3	90.9	1.86	3.38
4	85.7	2.29	5.06
5	78.2	2.82	7.59
6	68.0	3.46	11.4
7	55.3	4.26	17.1
8	42.2	5.24	25.6
9	30.5	6.44	38.4
10	20.8	7.93	57.7
11	13.6	9.75	86.5
12	8.8	12.0	129.8
13	5.6	14.8	194.6
14	3.6	18.1	291.9
15	2.3	22.3	437.9
16	1.5	27.4	656.9

RE. These data were prepared from preliminary curve shown in Figure 3 and used to prepare emulsion calibration curves shown in Figure 4.

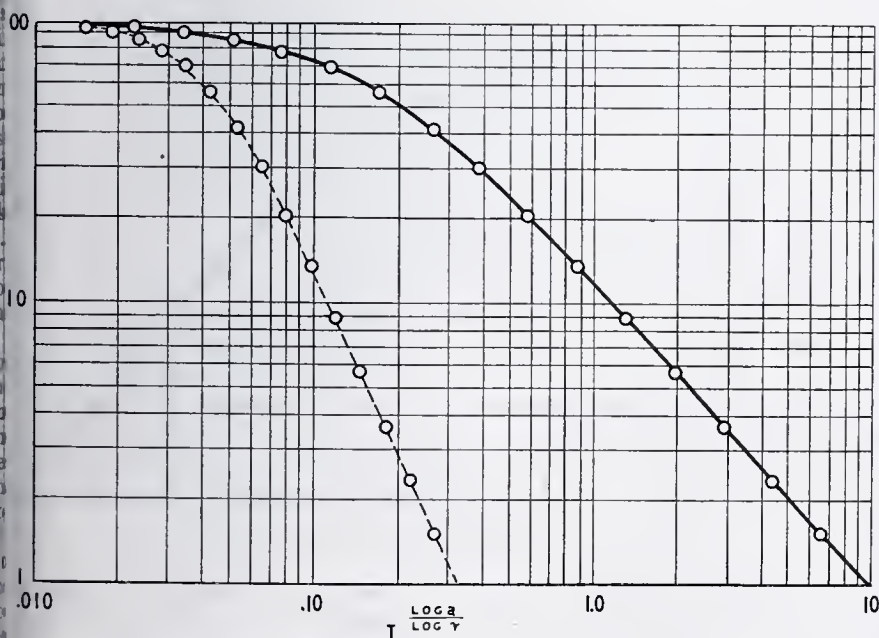


Figure 4. Emulsion Calibration Curves



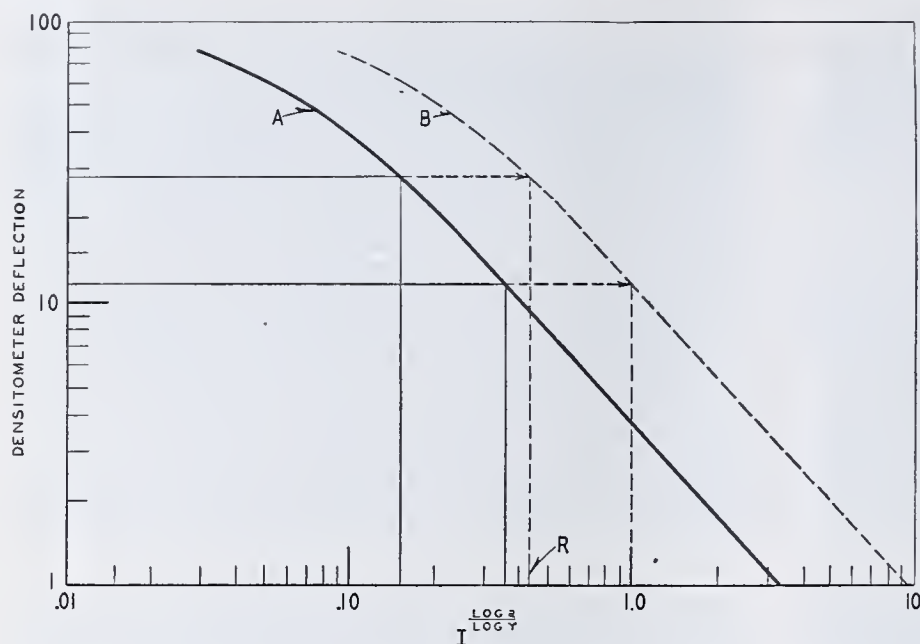


Figure 5. Calculation of Intensity Ratio from Emulsion Calibration Curve

with a logarithmic scale to calculate  $I_a/I_s$  values. The method of preparing this scale is shown in Figure 6.

The entire ordinate scale from deflections of about 3 to 95 is projected as shown in the figure for the six values, 5, 10, 20, 40, 60, and 90. The scale prepared as described above will be referred to as the "emulsion calibration scale", or simply as "the calibration scale", in further discussions. The calibration scale is used in slide rule fashion with the fundamental log scale to determine  $I_a/I_s$  values from deflection data. To do this, the two scales are adjusted with respect to one another, so that unity on the log scale coincides with the deflection for the internal standard line as read on the calibration scale. The value of  $I_a/I_s$  appears on the log scale at the point in juxtaposition with the deflection for the analysis line on the calibration scale.

The foregoing slide rule technique is practical only when the characteristics of the film used and the method of development and processing are sufficiently reproducible that the emulsion calibration curve does not have to be changed very frequently. This method was developed for use in the aluminum industry, where it has been found highly satisfactory from a standpoint of efficiency, accuracy, and convenience, particularly when the Dunn-Lowry calculator is used.

**PERIODIC CHECKING OF EMULSION CALIBRATION CURVES AND CALIBRATION SCALES IN TWO-LINE METHOD.** After the emulsion calibration curve or scale has been prepared and put into service, it should be checked at regular intervals. Two types of verification tests are suggested as a regular part of the analytical routine.

A series of measurements of the calibration pair should be made every day in regular production spectrograms. The data so obtained are referred to the emulsion calibration curve or scale and the fundamental log scale, and the apparent value of the intensity ratio is determined. If the result is greater or less than the intensity ratio (real or arbitrary) used in the original calibration, the response of the emulsion has changed, and if immediate corrective steps in photographic processing do not restore the original value of intensity ratio, a new calibration curve or scale is prepared.

A second type of verification test of greater rigor should be applied whenever the foregoing test indicates a change in contrast, when starting to use a new emulsion batch, or when a faulty calibration is indicated by inconsistencies among percentage scales. This test consists of preparing a series of spectrograms of the alloy used for the original calibration at three different intensity levels. The effective photographic speed of the spectrograph should be adjusted to yield low, medium,

and high microphotometer deflections for the three series of tests. The calibration pair is measured in all the spectra and the intensity ratios are determined. If the emulsion calibration curve or scale is still valid, the average ratio obtained in each of the three groups of reading will be equal to the original value.

In ordinary spectrographic work, the curve portion of the calibration curve is a function of the straight-line portion, as long as the same emulsion formula is used. Accordingly, the calibration scales obtained in the laboratory over a period of time may be classified according to the slopes of the straight-line portions of the curves and can be preserved for future use at such a time as tests indicate a slope represented among former curves.

**BACKGROUND CORRECTIONS.** In the foregoing procedure the effect of spectral background (continuum) was ignored. In most industrial procedures, the excitation conditions, the optical arrangement, and the exposure time are selected with a view towards keeping spectral background to a minimum, and in the most widely used spark techniques the background is kept sufficiently low to have no significant effect on analytical results.

When determining small amounts of certain constituents by either arc or spark techniques, excessive spectral background are sometimes unavoidable. In such cases the analyst has no recourse but to make a correction for background. Unfortunately, no rigorous method of background correction has been developed and whenever the background is sufficient to require correction the errors in analysis will be greater than when no background is present, regardless of the method of correction. The amount of background which may be tolerated without correction depends upon the absolute reproducibility of densities, the source of the background, the degree to which the photographic emulsion shows the Eberhart effect, and the accuracy required of the analysis.

Obviously, if the reproducibility of the densities of both lines and background is high, much larger amounts of background may be tolerated. If the background is caused by the major components of the samples and if its intensity is proportional to the intensity of the internal standard line, the systematic errors introduced by spectral background are the due to the Eberhart effect. Spark analyses are generally in the

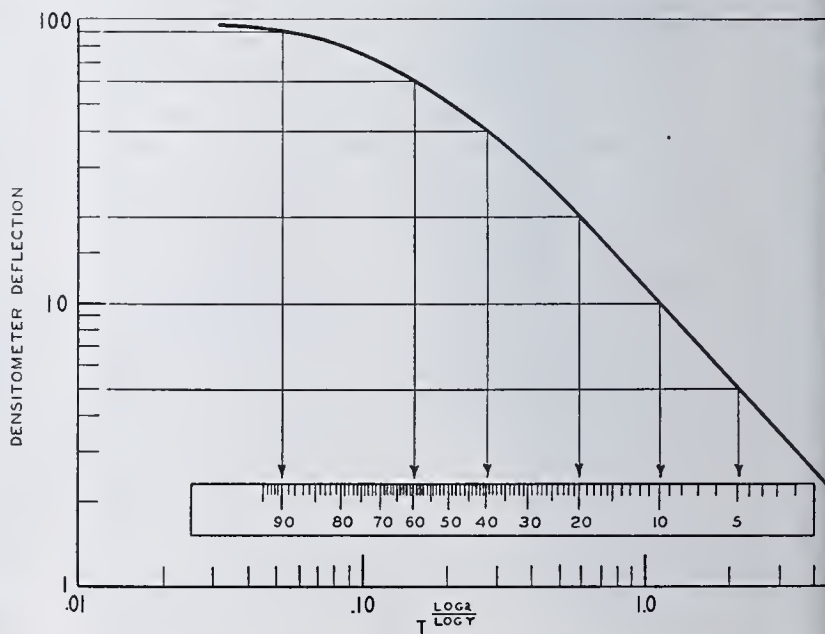


Figure 6. Preparation of Emulsion Calibration Scale



category and, if an emulsion and a processing technique which could eliminate the Eberhart effect were available, background corrections would be of no value in most spark work. Excessive background would still be undesirable, since the reduction in the sensitivity of the test by inclusion of background in the measurements would increase the random error of analysis. Background caused by the matrix or by the internal standard element can sometimes be expressed graphically as a function of the intensity of the internal standard and a correction applied which takes into account the Eberhart effect. This is accomplished by correlating the emulsion calibration curves of the continuum and the spectrum lines. The method can be applied to slide rule type calculations by the addition of a third scale providing the correction.

When the background is affected by variables which cannot be assumed to be a function of the intensity of the internal standard line, as often is the case in direct current arc techniques, a more rigorous correction method may be required. Many practical expedients have been employed, the most common being the following:

a. Calculation of "net densities" of the spectrum lines by using the deflection of the background adjacent to the line in place of  $I_0$ , the deflection for complete transparency in the conventional density formula

$$D = \log_{10} I_0/I$$

b. Adjusting the deflection to 100 for the background near the line (equivalent to a).

c. Numerically adding the difference in deflection between background and unexposed emulsion to the observed line deflection.

d. Converting the deflection of the background near the line to intensity by the use of an emulsion calibration curve prepared for a continuum and subtracting this intensity from the total intensity of the line plus background as determined from the conventional emulsion calibration curve.

None of the foregoing methods is rigorously correct and their use is justified only when the end results are found by experiment to be sufficiently accurate. Of the four methods given, *d* is generally more accurate, but it is cumbersome and time-consuming. A modification of this procedure is described by Strock (11). Strock also gives an excellent discussion of the problem of background correction as encountered in direct current arc work and while his method is too cumbersome for most high-speed, high-volume, industrial applications, it is probably the closest approach to a rigorous method of background correction.

In the foregoing discussion, only the so-called spectral background was considered. On some spectrographs, particularly on Littrow-type instruments, the plate or film may bear an appreciable amount of background caused by scattered light. Scattered light presents much the same problem as spectral background, except that, being polychromatic, it is even more difficult to correct for accurately. Both spectral background and background caused by scattered light are highly objectionable in excessive quantities, and since no method of correction completely eliminates the errors introduced, the best practice is to take all precautions to keep background to a minimum and to do everything possible to improve the absolute reproducibility of densities. Fortunately, in most metallurgical analysis and a large proportion of all spectrographic determinations of low concentrations, both spectral background and scattered light can be kept sufficiently low and absolute densities reproduced to such a degree that background corrections are unnecessary.

**WORKING CURVES AND PERCENTAGE SCALES.** Under the assumptions of the internal standard system the intensity ratio  $I_a/I_s$  for a particular alloy or material is a function of the single variable, concentration. Therefore, when the value of  $I_a/I_s$  has

been determined as described above, concentration can be determined if sufficient data on samples of known composition are available. The correlation of such data is generally accomplished graphically and, because of the nature of the mathematical relationship between concentration and the intensity function, it is convenient to use a log-log plot. Such a graph is called a "working curve". For purposes of this discussion the working curve is defined as a graph on which concentration expressed in per cent is plotted logarithmically on the ordinate scale against  $R$  plotted logarithmically on the abscissa scale.

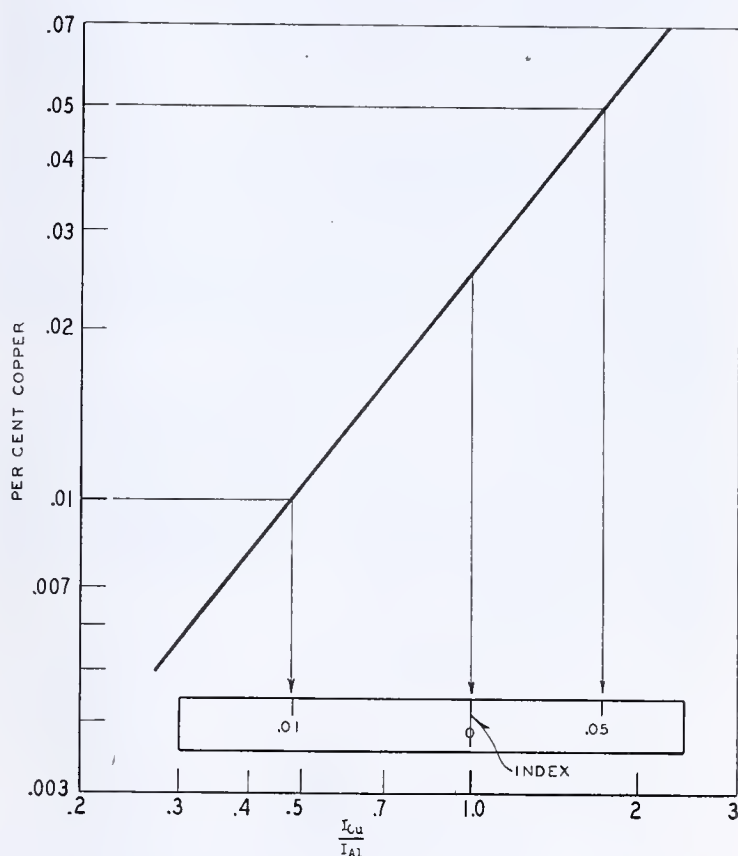


Figure 7. Projection of Initial Values on Percentage Scale from Typical Working Curve

Working curves are based on relatively large amounts of data obtained from standard samples containing known amounts of the element to be determined. All details of excitation and photography should conform to the practice to be used in analyzing unknowns. The arithmetic mean  $I_a/I_s$  value is used as abscissa, and the known percentage as ordinate in determining each point on the working curve. In many cases the working curve is a straight line within the ranges of concentration covered by a particular line pair. In most routine work the assumption of linearity is sufficiently valid. Self-reversal, failure to correct for excessive background at the analysis line, and the effects of increasing concentration on the behavior of the internal standard line each may contribute to curvature of the working curve. In the routine analysis of aluminum it has been found that the deviation from linearity is insignificant except when measuring very weak lines without making a background correction.

Once the working curve is plotted it can be used directly, for the determination of per cent constituent if  $I_a/I_s$  is known. However, a simpler and more rapid procedure is possible. In the determination of  $I_a/I_s$  by the use of either the emulsion calibration curve or the emulsion calibration scale,  $I_a/I_s$  is read directly from the fundamental log scale. Since concentration is assumed to be a function of the single variable  $I_a/I_s$ , it is obvious that a concentration scale bearing an index mark to denote the location of unity on the fundamental log scale can be used in place of the log scale. Since the concentration corresponding to each value



of  $I_a/I_s$  can be read from the working curve, the preparation of this scale is easily accomplished. The procedure is as follows:

Consider the typical working curve shown in Figure 7. To prepare a percentage scale from this curve, all percentage readings can be projected from the ordinate scale onto the percentage scale, as shown for 0.01 and 0.05%. This procedure can be carried out precisely as described for the preparation of the emulsion calibration scale. However, when the working curve is a straight line, the following simpler and more rapid procedure is used:

Project only two percentages on the scale, selecting percentages near the two extremes of the working curve. Also mark the scale with the index line (the point corresponding to  $I_a/I_s = 1$ ). Remove the scale from the calculating board and place it on semi-logarithmic or bilogarithmic paper in such a position that the marks indicating the two projected percentages on the scale intersect corresponding coordinates on the graph paper, as shown in Figure 8. In this figure the scale on which 0.01 and 0.05 were located in Figure 7 is placed on the graph paper in such a position that the 1 and 5 coordinates intersect the scale at the marks corresponding to 0.01 and 0.05% copper. The scale is then completed by marking all the desired percentage divisions and subdivisions at the appropriate intersections of the scale with the logarithm coordinates: 0.02 is marked at the point where 2 intersects the scale, 0.04 at 4, 0.06 at 6, etc. While semilogarithmic paper is used in Figure 8, it is obvious that the horizontal coordinates could have been linear, logarithmic, or any other type of scale, or might have been omitted altogether, since only the vertical coordinates were used.

The scale prepared as described above is generally referred to as a working scale or percentage scale, and the latter designation is used in this paper. Before considering the actual use of the percentage scales in mechanical methods of calculation, it is necessary to take into account the troublesome effect known as "curve drift" or "scale drift", which is encountered in many spectrographic methods.

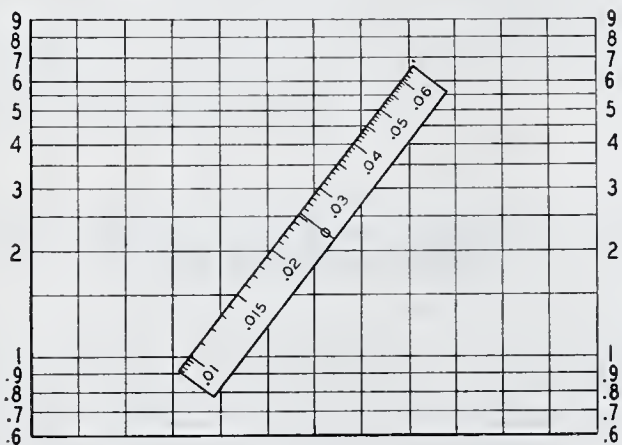


Figure 8. Preparation of Final Percentage Scale

**CURVE DRIFT OR SCALE DRIFT.** In the internal standard system it is assumed that the intensity ratio is a function of the single variable intensity ratio. If this were rigorously true, a permanent standardization could be made and no further standard spectra would be required in a routine application. Unfortunately, however, there are many variables in present-day procedures other than concentration which affect the measured intensity ratios. As a result, working curves and percentage scales show a slight irregular drift and it is necessary to run frequent standard samples to correct for this drift. While all the causes of drift are not understood, it is known that a part of the drift is caused by an actual change in intensity ratio and a part is caused by errors in measurement of intensity ratio. The principal factors affecting the actual intensity ratio produced by the spark are atmospheric conditions and gradual deterioration in apparatus. The apparent drift caused by errors in measurement are largely introduced by variables in the photographic process. The photographic errors are caused by inherent, systematic non-uniformities introduced by methods of manufacture, by the

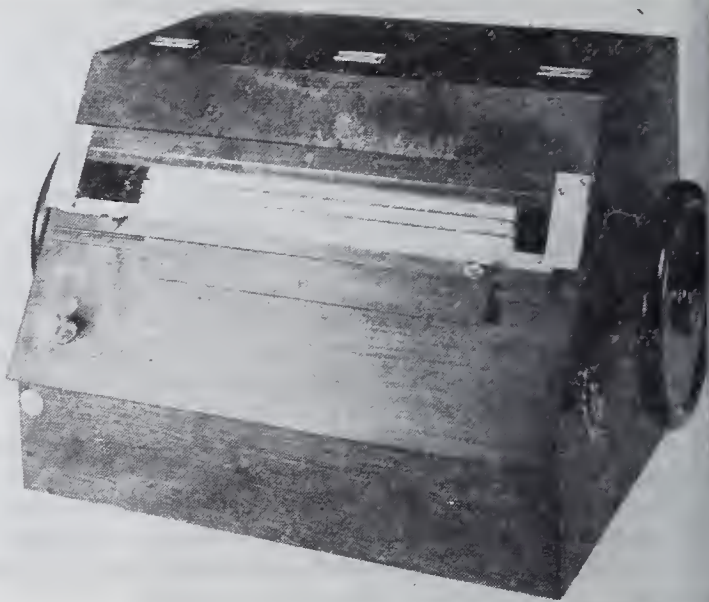


Figure 9. Dunn-Lowry Calculator for Spectrographic Analysis

rather large effects of humidity on the emulsion, by changes in relative response of the emulsion to different wave lengths, and systematic errors resulting from the use of commercially available developing machines which develop rather reproducibly but not uniformly. A small but significant portion of the apparent curve drift is caused by defects in emulsion calibration in large-scale routine work.

Curve drift is a serious problem in the analysis of aluminum alloys but almost negligible in the analysis of iron and steel. This difference is due largely to the differences between the spectra of iron and aluminum. The aluminum spectrum contains so few lines suitable for use as internal standards that it is necessary to use line pairs of widely different characteristics and widely different wave lengths in routine analysis. Add to this troublesome surface effects caused by the formation of aluminum oxide on the electrode surface and it is apparent that the analysis of aluminum presents problems not to be expected in steel or many other materials. The use of dissimilar line pairs and line pairs differing substantially in wave length magnifies most of the inherent defects of the internal standard method and invalidates several of the fundamental assumptions of the internal standard method. Moreover, the experimental errors introduced by variations in excitation, photography, development, and densitometry are magnified. The apparently greater curve drift encountered in the analysis of aluminum is only a manifestation of the failure of the internal standard method resulting from the necessary violation of some of its cardinal principles.

It has been found experimentally that the net effect of curve drift can be treated as a proportional change in all intensity ratios for a particular line pair. Accordingly, the necessary correction can be made by applying a factor to all intensity ratios. This fact was established empirically by experimental methods and applies only to the normal shifting of working curves under carefully controlled conditions with adequate emulsion calibration, and only within the relatively small range (two- to threefold on a concentration basis) over which a particular line pair is used in the routine analysis of aluminum alloys. Correction for curve drift is then essentially merely the determination of the index—that is, finding of the concentration at which the intensity ratio equals unity. The routine procedure for determining the index is given in the following sections on mechanical calculation.

**THE DUNN-LOWRY CALCULATOR.** The Dunn-Lowry calculator (Figure 9) is an adaptation of the "squirrel cage" slide rule developed by Aluminum Company of America for routine spectrographic calculations.



It consists essentially of a cylindrical drum bearing the percentage scales for the various elements and alloys, and a rule bearing the emulsion calibration scale. The percentage scales are mounted on wood or metal rules whose faces form the periphery of the cylinder. Each rule can be moved laterally to compensate for index shift. The calibration scale is so arranged that it may be made to slide laterally with respect to the drum and is so positioned that any percentage scale on the drum can be brought into juxtaposition with it by rotating the drum. An index line is inscribed on a small glass or plastic tab permanently fastened to the window frame of the calculator. This index corresponds to an intensity ratio of unity and all percentage scales are adjusted with reference to this point before use. This line will be referred to simply as the index line.

After the calibration scale and the percentage scales have been prepared as previously described, the first step in the use of the calculator is the index adjustment of the percentage scales. To determine the index of a particular percentage scale, the following procedure is followed:

Measure the analysis and internal line photometrically in a series of spectrograms of a standard containing the same order of magnitude of the element to be determined as expected in the samples to be analyzed. (Two or more standards of different composition may be used without altering the subsequent procedure.) Set the percentage scale so that the expected index is near the index of the calculator, but do not move the percentage scale again until the final index is determined. For each spectrum, adjust the calibration scale so that the deflection for the analysis line falls in juxtaposition with the known concentration on the percentage scale, and record the concentration reading coinciding with the deflection of the internal standard line. This percentage is the percentage at which the intensity ratio is unity and the average of the percentages so determined for all of the spectrograms is the "index". Slide the percentage scale until the index so determined coincides with the index line on the calculator. For the continual index adjustment of a percentage scale in regular use, the half-correction method is used. This method consists of taking as the final setting of the index the arithmetic mean of the index as determined immediately before the analysis, and the index as used during the immediately previous analyses. This method is used in all Aluminum Company of America laboratories in a continuous routine operation.

The per cent constituent on an analysis sample is calculated on the Dunn-Lowry calculator by simply adjusting the calibration scale so that the deflection for the internal standard falls at the index mark, and reading per cent constituent on the percentage scale at the point which falls in juxtaposition with the deflection for the analysis line. When more than one determination on a particular sample is referred to the same internal standard line, the calibration scale is adjusted only once and the various elements are calculated by bringing the appropriate percentage scales successively into view on the calculator.

**MULTIPLE DETERMINATION CALCULATING RULE.** The multiple determination calculating rule is a type of slide rule calculator developed by Aluminum Company of America for use with recording microphotometers in routine analysis.

The rule is designed for use on a special calculating board consisting essentially of a drawing board equipped with a movable horizontal bar which will move up and down on the board but will not move sideways. This bar is equipped with bearings, guides, or a pulley arrangement, so that it will remain parallel to the front edge of the board at all times. A rectangular opening, approximating the dimensions of the largest microphotometer chart likely to be encountered, is cut in the central portion of the board. A glass plate is inserted in this opening, flush with the top surface of the board. The plate is illuminated with fluorescent type incandescent tubes from below. The calibration curve is fastened semi-permanently on this glass plate and charts representing analyses are superimposed on this chart. The horizontal rulings on the chart and the abscissa coordinates of the calibrating curve

are made parallel to the bar on the calculator by aligning the chart and curve with suitable reference marks. It is desirable that the calculator be equipped with a clamping mechanism to hold the chart paper in place. Otherwise, scotch tape or thumb tacks are used.

A photograph of a multiple calculating rule mounted on a calculating board is shown in Figure 10. This calculating rule is equipped with a replaceable nest of scales. A separate nest of scales is used for each different alloy analyzed. The front edge of the rule is metal and is close to the surface of the board. A vertical hairline is ruled on the glass slide at a position corresponding to the pointer which rides along the edge of the rule. The individual percentage scales are adjustable with respect to each other and with respect to the index mark inscribed on the edge of the rule.

To determine the correct location of a percentage scale with respect to the index mark on the rule—i.e., to determine the index of the percentage scale—the following procedure is used:

Measure a series of spectrograms of a suitable spectrographic standard. Superimpose the chart paper on the calibration curve, taking care to align the chart with the reference marks indicating the ordinate scale on the curve. Move the rule vertically until its edge intersects the deflection peak representing the element to be determined. Move the rule laterally and move the slide bearing the cross hair until the pointer intersects the calibration curve and the hairline crosses the percentage scale at the known percentage of the element sought. Move the rule vertically (taking care not to move it horizontally) until the edge matches the deflection peak for the internal standard. Adjust the cross hair slide so that the pointer again intersects the curve. Record the percentage now indicated by the hairline. The average of the percentages so determined for a number of standards is taken as the index for subsequent analyses. The percentage scale is adjusted so that the index determined falls at the cross hair when the pointer coincides with the index line on the edge of the rule. At least four exposures should be used to establish an index originally. When the percentage scales are used at very frequent intervals, the half-correction system (described previously) is employed.

To determine percentage composition on unknowns, proceed as follows: Move the rule vertically until its edge passes through the deflection peak for the internal standard. Adjust the rule laterally until the index mark on the edge of the rule intersects the calibration curve. Move the rule vertically (with no horizontal movement) until the edge of the rule coincides with the deflection peak of the analysis line. Move the cross hair slide until the pointer intersects the curve, and read the analytical result on the appropriate percentage scale. Of course, if more than one constituent is to be determined with reference to the same internal standard, the rule is adjusted laterally with respect to the internal standard deflection only once, and the various

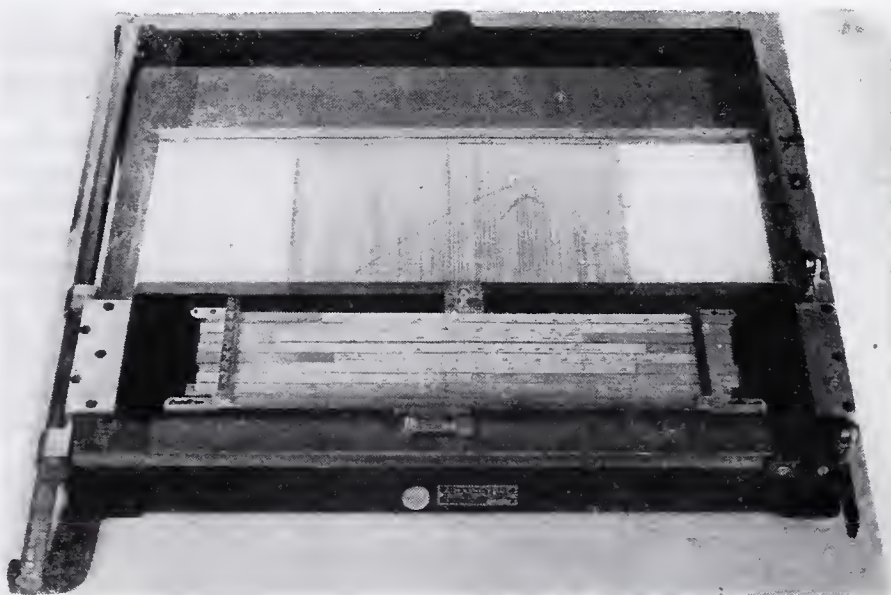


Figure 10. Multiple Determination Calculating Rule Used with Recording Microphotometers



elements are determined successively by simply moving vertically to the deflection peaks and reading percentages on the appropriate scales.

#### SUMMARY

The techniques discussed have been those developed specifically to meet problems encountered in the routine analysis of aluminum. While an attempt has been made to treat the subject from a broader viewpoint than that of a spectrographer specializing in aluminum alloys, the emphasis has naturally been greater on those problems which are most serious in aluminum analysis. The specific methods of analysis best suited for one type of material usually cannot be transferred to another type without some modification, but the underlying principles involved in all spectrographic analyses are much the same, and the problems encountered differ in degree but not in kind.

It may seem that an overly critical attitude was taken towards commercially available apparatus. It was the deliberate intention of the author to emphasize the defects of existing equipment for the double purpose of helping prospective users in the selection of apparatus and in offering constructive criticism which may aid the manufacturers of apparatus in meeting the practical requirements of routine spectrography. Emphasis on the limitations and sources of error in the spectrographic procedure was also intentional. The overly euphemistic treatment of spectrographic procedures characteristic of much of the literature in recent years no longer serves any useful purpose. No longer is it necessary for spectrographers to act as salesmen for their art or the apparatus they use. Frank recognition of the imperfections and limitations of existing techniques will not only aid in relegating it to its proper position in the analytical scheme but will foster developmental work aimed at improving these techniques. It is the opinion of the author that the most important advances in routine spectrographic analysis will come through the improvement in or elimination of the photographic films and plates and in the development of improved excitation sources. Free ex-

change of information along these lines and a critical appraisal techniques and apparatus in the literature will bring the fundamental improvements now under development to earlier fruition.

#### ACKNOWLEDGMENTS

The foresight and constructive guidance of H. V. Churchill, chief chemist of the Aluminum Company of America, have been large factors in bringing spectrographic analysis to its present status in the aluminum industry. The author also gratefully acknowledges the contributions of the laboratory staff of Aluminum Research Laboratories and of the men and women of the plant laboratories of the Aluminum Company of America, whose collective experience this paper is based. Finally, a special acknowledgment is offered to the manufacturers of spectrographic equipment for their splendid cooperation and assistance in making possible the great expansion of spectrographic analysis during the present emergency.

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# Emission Spectrographic Equipment Used in Quantitative Analysis

## Proposed Minimum Requirements

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**S**PECTROGRAPHIC analysis definitely has established itself as an important control and testing tool in industry. Hundreds of commercial spectrographic laboratories have been established. Colleges and universities are offering courses in spectrographic technique. This excellent progress may be traced to the initiative of many academicians in the field of applied physics and to manufacturers of spectrographic equipment who at an early stage in the development of the field designed and provided equipment for others not in a position to build such apparatus for themselves. The spectrographer, although he has also contributed his share to the advancement of spectrography, is extremely grateful to these early pioneering spectroscopists and manufacturers for the foundation established in this new and interesting field of applied science.

The present-day spectrographer defines himself as one who applies spectrographic equipment to analytical problems in the laboratory and thus differs from the spectroscopist who may design such equipment or may be concerned with the theoretical

concepts of spectra. A spectrographer who has been trained in chemistry may prefer to call himself a spectrochemist, but for the purpose of this paper he is included in the former category.

The spectrographer does not claim to be an expert in all the fields that are involved in spectrographic analysis—i.e., photography, optics, electronics and electrical circuits, general chemistry and physics, etc. He may have specialized in one of the fields prior to entering spectrography, but any necessary knowledge of the other fields is usually acquired as further experience is obtained. However, on comparing notes with other spectrographers, on studying numerous publications, and on experimenting in the laboratory, he finds one question which he cannot answer to his satisfaction—what is to be expected of commercial spectrographic equipment?

#### REASONS FOR MINIMUM REQUIREMENTS

Although no adopted minimum standards exist for emission spectrographic equipment in general, the spectrographer finds



himself in a field where enough literature has accumulated to justify an index of subject matter (2) and a compendium of operating conditions (1). Enormous publicity has popularized the field of spectrography through layman periodicals, commercial advertising, conferences on spectrographic analysis, and technical journals covering fields as far apart as biology is from metallurgy. The employer of the spectrographer reads of these events and his enthusiastic expectation for results of high precision and accuracy may overwhelm him, especially after appropriating thousands of dollars for his spectrographic installation. Such a condition may place the spectrographer in a "do or die" situation if his results are adverse. However, no matter how excellent the technique of the spectrographer may be, his results cannot be any better than the merits of his equipment permit.

The spectrographer, whose vocation in most cases has been created by the joint efforts of the field of physics and the manufacturers of spectrographic equipment, has now reached a stage in his own development where he is getting bold enough to turn to his "creators" and demand specific minimum standards for his much-publicized equipment to protect his own interests. The offspring definitely is growing up!

The AMERICAN CHEMICAL SOCIETY has high standards for chemicals of reagent grade. The American Society for Testing Materials has a multitude of requirements in effect for apparatus used for testing purposes. Many other technical groups have such requirements. It is logical that the equipment of the spectrographer be covered by similar standards.

The spectrographer does not request complete standardization of equipment at this time, although further standardization would permit a more common spectrographic language. Standardization may be the result of future development work. However, the field is still fertile for competition between manufacturers of spectrographic equipment and for difference of opinion between spectrographers concerning who has, or what is, the best spectrograph, densitometer, or excitation unit, for a given application. Nevertheless, this variety of opinion concerning major issues in the spectrographic field still does not eliminate the necessity of establishing minimum standards for any piece of emission spectrographic equipment.

The spectrographic equipment manufacturer should not be required to work out the details of a given analysis. Most of the development work has to be left to the ingenuity of the individual spectrographer. However, the presence of a representative of the equipment manufacturer during the early period of a new installation has proved extremely helpful to the spectrographer. This period could be used advantageously in aiding to check the equipment thoroughly for its ability to meet adopted minimum standards.

It is realized that of the many mass production parts of which modern spectrographic equipment is composed, all may not meet absolute perfection. Such a condition may cause occasional difference in performance of units of the same type and model. However, the assembled equipment should at least be capable of meeting certain minimum requirements, so that the spectrographer more easily may isolate errors due to technique from those arising from possible defects in the apparatus. If the equipment continued to meet the minimum standards with the accepted techniques, the spectrographer could check his own variations of the techniques thoroughly before complaining to the equipment manufacturer about adverse results. Thus, the latter also would be protected by the requirements.

Since the field of spectrography has grown more rapidly than the ability to train personnel for handling its possibilities, many lengthy requisitions and procurement specifications have been written by individuals whose knowledge of the field was not established until after the apparatus was bought and paid for. This has been especially true during the present emergency when rapid means of analysis had to be installed in a short period of

A proposal is made to establish minimum requirements for equipment used in precise and accurate emission spectrographic analysis with the view of stimulating discussion toward the development of final minimum requirements. A discussion of reasons for the requirements is given, followed by a discussion of the nature of such requirements. Proposed minimum requirements for the spectrograph, densitometer or microphotometer, and excitation equipment are presented. The accepted minimum precision of the final results is given in terms of an empirical relationship between the average deviation and the level of concentration. The conditions under which the minimum precision is determined are given. The requirements emphasize the performance of the equipment rather than detailed construction of apparatus.

time. If only for the benefit of these newcomers, guiding minimum requirements are justified.

Specifications for spectrographic equipment should include not only description of parts with physical dimensions and diagrams of electrical circuits, but also the nature of results to be obtained, expressed in terms of universally adopted factors of precision and accuracy. When a chemist orders an analytical balance, the size of the pans and length of the beam are by far subordinate to the sensitivity and reproducibility obtained with the instrument. A spectrographer should have a similar attitude toward his equipment.

It should not be interpreted that the writer has had difficulties with equipment on all points covered by the proposed requirements. On the contrary, personal experience with commercial equipment plus the knowledge gained by visits to numerous other spectrographic laboratories, many of which used equipment made by different firms, proves that in general the commercial instruments have eliminated many previous difficulties and have paved the way for higher standards. Only for the reasons given are the requirements being considered.

It is one thing to advocate minimum requirements and quite another thing actually to present them. However, it is expected that the reader will accept this effort in the spirit of keeping the standard of spectrographic analysis on a high plane.

#### NATURE OF MINIMUM REQUIREMENTS

The minimum requirements should be practical, brief, and as simple as possible. They should apply to emission spectrographic equipment in general and should not emphasize any one type, mounting, or optical system. Thus, the requirements should avoid any reference to spectrographic topics which are problems of applied technique and personal choice—i.e., the prism versus the grating, the electrical characteristics of the excitation, the film versus the plate, the type of photographic emulsion, and the type of counter or supporting electrodes. Unless lengthy and cumbersome procedures are needed to overcome defects of a given instrument, a condition which is rare if not extinct, the emphasis should be on the nature of the readings or recordings expressed in terms of precision rather than on the details of the mechanism which produced the readings or recordings.

Sawyer and Vincent have presented a paper on "Specifications and Testing of Spectrochemical Apparatus" (3). Any adopted requirements or purchase specifications should be supplemented by a study of their paper. However, an inexperienced spectrographer may hesitate to apply portions of its contents to test his new equipment, because of his own temporary lack of knowledge of the optics involved. The method suggested by Sawyer and Vincent to determine errors which arise in spectrochemical analysis, in this writer's opinion, would be a means to check the accuracy of results reported by a so-called established spectrographic



laboratory, operated by an experienced spectrographer who has mastered the equipment in question. Its value in establishing requirements for new equipment would depend on how rapidly a spectrographer could learn to appreciate the significant difference between his systematic, and accidental or random errors. Although it may not have been the intention of Sawyer and Vincent in their specifications and tests, one of the purposes of the present proposal is to give to the technician who is inexperienced in spectrography minimum requirements which can be incorporated practically verbatim into a purchase order requisition for emission spectrographic equipment. The proposed requirements are given toward the end of this paper. Following is a discussion concerning their nature:

*Nature of Requirements.* *a.* It is admitted that the requirements for the spectrograph may be considered as vague. Differences in dispersion, linear and nonlinear, with corresponding degrees of resolution, are available, since there is a variety of mountings and optical systems provided in commercial spectrographs. The choice of instrument thus depends on its application to a given type of analysis. This portion of the requirements is also influenced by the opinion of the writer, in that the spectrograph itself is considered third in importance after the excitation equipment and densitometer in relation to precise and accurate quantitative analysis. Any defects in the spectrograph are usually of a constant nature and even these, although covered by the requirements in a general manner, have been eliminated or diminished to an insignificant minimum by good design—i.e., lengthy exposure times, scattered and stray light, ghost lines, and light leakage.

*b.* No reference is made in the spectrograph requirements to obvious incorporations such as a variable slit width, or a racking camera.

*c.* A means of spectrum line identification has been included in the densitometer or microphotometer requirements, since any line must be identified before being evaluated. Whether this means of identification is physically part of the densitometer or microphotometer or contained in some auxiliary apparatus is of no concern of the requirements. The use of exposed wave-length scales on photographic emulsions, or the projection of iron spectra, wave-length scales, commercial master plates, and known spectra master plates made by the spectrographer, depends on the complexity of the unknown spectra, the number of lines to be identified, and the magnification required to detect the necessary details that can be resolved by the dispersing medium of the spectrograph.

*d.* The reproducibility of all readings or recordings and corresponding indicator settings has been emphasized in the densitometer or microphotometer requirements. The maximum deviation is used instead of standard deviation for purposes of simplicity and in place of average deviation to assure that the reading or recording, any one of which may represent a single quantitative analysis to be reported on the basis of the given reading or recording, does not fall out of the desired range.

*e.* It is realized that some densitometers or microphotometers use a log density scale rather than a linear transmission scale. In these cases it would be necessary to convert to the linear form for the purpose of checking the proposed requirements.

*f.* The two terms "reading" and "recording" have been used throughout to include both the nonrecording and recording types of instrument. The terms "densitometer" and "microphotometer" have been used together in the requirements without exception for the benefit of those firms which prefer to describe their instrument as one or the other.

*g.* The minimum requirements for the excitation equipment are considered after specifying the nature of the other units, since the performance of the excitation equipment is "registered" by the spectrograph and "examined" by the densitometer or microphotometer. Thus, the final precision test, although listed under the excitation equipment requirements, should be interpreted as

including any lack of precision inherent in the other units. It is assumed, however, that if the spectrograph and densitometer or microphotometer meet their respective requirements by the results of tests which are to a great degree independent of the merit or defects of the excitation equipment, and if the technique is of satisfactory quality, most of any remaining lack of precision in the final result can be traced to errors in the light source. Therefore, it is believed that the inclusion of final precision and accuracy tests under "Excitation Equipment" is justified—especially when the requirements describe the excitation equipment as being used in conjunction with other units which already are specified.

*h.* The specifications for the intensity of the light source, as suggested by Sawyer and Vincent (3), have been included in the requirements for the excitation equipment.

*i.* The precision also has been emphasized in the excitation equipment requirements. Reproducibility tests for excitation units many times are questionable, owing to lack of knowledge concerning the homogeneity of the samples. This situation is further complicated by the fact that in most cases there are no accepted independent means for checking homogeneity. However, any establishment that confidently cannot produce a reasonably homogeneous sample for quantitative tests should not consider spectrographic equipment. Such a condition should be decided prior to any installation, by the establishment submitting samples to the equipment manufacturer or to other spectrographic laboratories having available techniques for reproducibility tests using the type of equipment and material in question. It is realized that reproducibility is a function of technique as well as the precision of the equipment. Thus, the homogeneity tests should be made with established technique having acceptable reproducibility. If such techniques are not available for the analysis in question, an agreement should exist between the establishment contemplating the use of spectrographic equipment and the manufacturer of such equipment whereby either the latter agrees to develop a reproducible technique for checking sample homogeneity or the former purchases the equipment at his own risk, leaving problems of sampling and reproducibility to his own research and development. Regardless of the nature of such an agreement, a sample is either a representative specimen for spectrographic analysis or it is not. There is no compromise.

*j.* Any one given magnitude of a numerical value to express acceptable precision covering all concentration ranges encountered in spectrographic analysis would be questionable if not impossible to attain. A spectrographer checking his precision in very low concentration ranges may take pride in his low average deviation from the mean in percentage concentration of the element in the sample, but his per cent average deviation may be extremely high. (The per cent average deviation is the  $\frac{\text{average deviation}}{\text{mean result}} \times 100$ .) Likewise, a spectrographer checking

precision in a comparatively high concentration range may enjoy his low per cent average deviation, but his high average deviation from the mean in percentage concentration of the element in the sample may justify the substitution of the wet-analysis technique for the spectrographic method.

It is realized that by choosing proper analytical lines, the accidental errors occurring in spectrographic analysis tend to have a constant percentage relationship to the quantity to be determined, regardless of its amount. However, a 10% average deviation may be acceptable precision at a concentration level of 0.1% but would not be acceptable at a concentration of 5.0% when competing with other independent means of analysis. In order to justify the use of spectrographic analysis at higher concentration levels, lower per cent average deviations are required to obtain acceptable precision. Therefore, in the proposed requirements an empirical relationship has been established between the minimum accepted precision and the concentration level where higher average deviations are permitted and lower per cent



average deviations are required for higher concentration ranges. This relationship also is in accord with the fact that in spectrographic analysis, as in other methods of analysis, lower relative precision is required in determining small constituents than large ones, at least in part because of the relation of sampling technique and constituent homogeneity limitations at extremely low concentrations.

At attempt has been made to adjust the empirical relationship to actual experience and to reports in general spectrographic literature suggesting what minimum requirements should be expected for equipment to give the usual claimed precision for concentrations between 0.0001 and 5.0%. The evaluations in the empirical relationship also were influenced by the usual precision obtained by the independent wet analysis—a method which may be substituted if the spectrographic analysis fails to equal or improve the precision obtained with the chemical technique.

Since there appears to be an appreciable difference in techniques used for concentrations below approximately 0.05% as compared to conditions used above this figure, the empirical relationship is divided into a low and medium range. Techniques calibrated for the medium range cannot claim the degree of precision at their lower concentrations as would lower range techniques specializing in concentration ranges which the medium range overlaps. The extreme lower concentrations of the medium range are usually considered merely as residuals, while the lower range technique claims a higher degree of precision for the same range of concentrations. No effort is made to cover the high range concentrations—i.e., above 5%—in this paper.

Many spectrographers will claim more precise results than required by the empirical relationship, especially in the low range. However, it should be realized that the precision specified here is minimum requirement. It is expected that improvements in technique may lead to more precise work.

Some spectrographers may desire a more statistical approach to precision by adopting the standard deviation rather than the average deviation. Such a modification may be an improvement. However, it is believed that the suggested empirical relationship could be comprehended more easily in terms of the average deviation.

k. Through personal experience and by contact with other spectrographers, it has been found that many in the field experience shifting of analytical working curves without any known change in technique. It becomes necessary to run a series of standards to determine the nature of the shift and make corrections accordingly. The reason for this shifting is not usually known. The use of small correction factors may not be inconvenient if the precision would remain satisfactory, but any extreme shifting of curves requiring abnormally high correction factors would indicate a poor day-to-day accuracy. If there has been no change in technique, the shifting must be due to some instrumental variation, though the source of variation may be unknown. The requirements given attempt to cover adequately any such shifting of analytical working curves.

l. No reference is made to requirements for photographic processing apparatus or calculating mechanisms, as both are assumed to be classified in the category of technique.

m. The minimum requirements have been written primarily for those control and testing laboratories requiring precise and accurate routine quantitative analysis. There may be some laboratories whose work involves only qualitative analysis and where a densitometer or microphotometer would not be used. Other establishments may require results of only a semiquantitative nature where the requirements for the densitometer or microphotometer and excitation

equipment could be less rigid. Such cases should be covered by mutual agreement and understanding.

With references of this discussion in mind, the following proposed requirements have been written. The small letters following the subtitles refer to parts of the above discussion.

## PROPOSED MINIMUM REQUIREMENTS

### I. THE SPECTROGRAPH (*a, b*)

1. The spectrograph shall give ample dispersion, resolution, definition, and wave-length range of spectrum lines to accomplish the given analysis. Where possible, the actual values for the required dispersion, resolution, and wave-length range shall be obtained from spectrographers specializing in the field of the given analysis.

2. The absorption of light in the process of dispersion shall be sufficiently low to permit exposure periods of 2 minutes or less, as further specified in III, 2.

3. The optical system shall consist of high-grade materials designed to keep phenomena such as scattered and stray light, production of ghost lines, and deterioration of optical parts at a minimum.

4. Light leakage, as noted by the fogging of highly sensitive photographic emulsions, shall be eliminated.

5. The construction and mounting shall be substantially rigid to permit permanent alignment of all optical parts.

### II. THE DENSITOMETER OR MICROPHOTOMETER (*c, d, e, f*)

1. A positive means of spectrum line identification shall be provided to accomplish the given analysis.

2. The densitometer or microphotometer shall provide a linear relationship between light transmission and indicator response, so as to permit uniform sensitivity between 0 and 100% transmission. A means for checking the linearity of the instrument shall be provided.

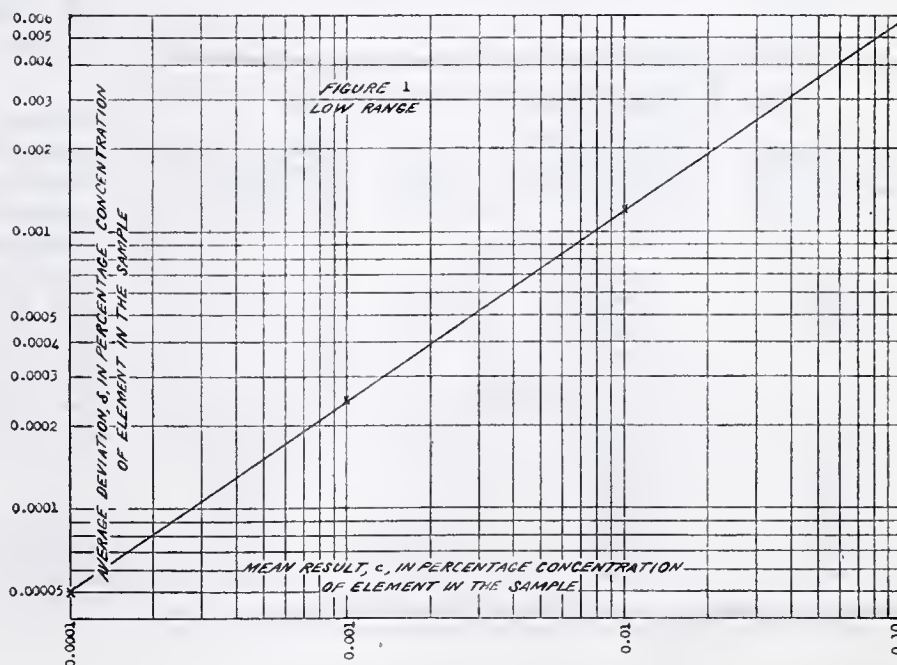
3. The densitometer or microphotometer reproducibility while reading or recording within one spectrogram on a given photographic emulsion shall include the following conditions:

a. Five check readings or recordings on any one of the uniform, adopted spectrum lines shall fall within a maximum deviation of 0.2% of full linear scale deflection without intermediate adjustment of the full-opaque setting or the clear emulsion setting.

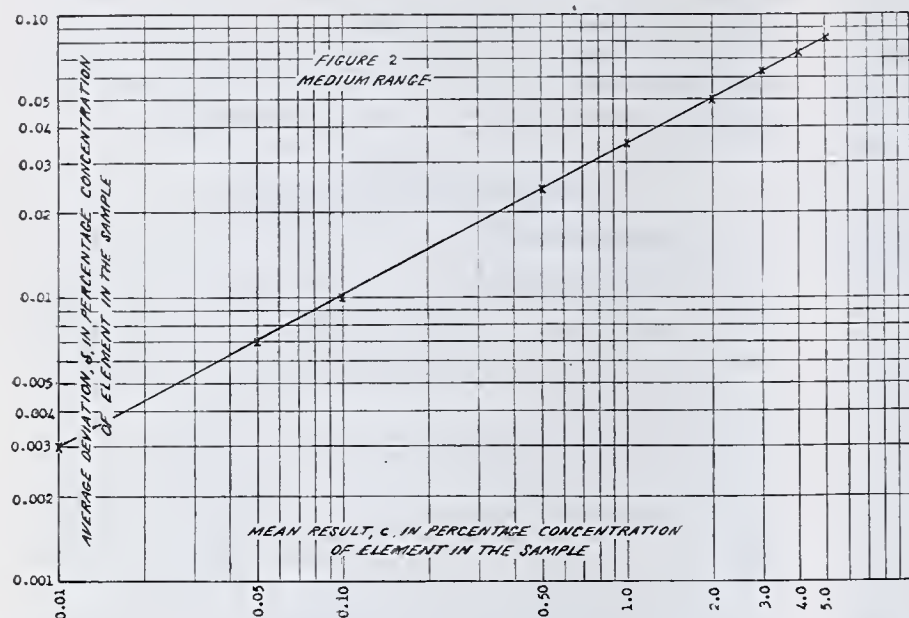
b. The full-opaque setting and the clear emulsion setting shall be repeated with the degree of precision required to permit the maximum deviation in line readings or recordings as specified in part a of this section.

### III. THE EXCITATION EQUIPMENT (*g, h, i, j, k*)

1. The sensitivity of the excitation equipment as determined in conjunction with a spectrograph as specified in I shall be such that the desired minimum concentration of the element or elements in question shall be detected; or the minimum possible concentration of the given element or elements to be detected shall be known to the spectrographer.







2. The excitation equipment used in conjunction with a spectrograph as specified in I shall produce a light source of sufficient intensity to provide an adequate exposure on standard photographic emulsions, without a condensing lens (a lens may be used with astigmatic instruments in those wave-length regions only where long focal distances would be inconvenient), in not over 2 minutes, with the source 10 inches or more from the slit of the spectrograph (see I, 2). (This requirement is to be used for testing the intensity of the light source, and with part I, 2, the speed of the spectrograph, and does not imply that established routine techniques have to be bound by the conditions given.)

3. The resulting accepted precision using the excitation equipment in conjunction with a spectrograph as specified in I and a densitometer or microphotometer as specified in II shall be determined as follows:

a. The accepted precision in the determination of any one element shall be expressed as the average deviation,  $\delta$ , in percentage concentration of the element in the sample, from the mean result,  $c$ , of eight or more individual trials. The highest accepted value of  $\delta$  shall depend on the magnitude of  $c$  in percentage concentration of the element in the sample, as given in the empirical relationship between columns 1 and 2 of Table I. The medium range should not be used for techniques specializing in concentrations of less than 0.1%. The precision may be described further as per cent average deviation as given in column 3 of Table I, and defined as  $\frac{\delta}{c} \times 100$ . For intermediate values

of  $c$  and  $\delta$ , see Figures 1 and 2, where the empirical values of  $\delta$  in Table I follow within close approximation a straight line when plotted against  $c$  on the given logarithmic axes. (For those who desire a mathematical expression for the empirical relationship

as given by the curves in Figures 1 and 2, the equation

$$\log \delta = \log b + n \log c \text{ or } \delta = bc^n$$

may be used, where  $\delta$  is the average deviation from the mean in percentage concentration of the element in the sample,  $b$  is the log axis intercept when  $c$  is unity,  $n$  is the slope, and  $c$  is the mean percentage concentration of the element in the sample.)

b. The individual results used to determine the value  $\delta$  shall be obtained from an analytical working curve based on a technique as specified in III, 2, c, and on standard or routine samples of accepted, known concentration meeting the homogeneity requirements in III, 2, d.

c. The analytical working curve and the value of  $\delta$  shall be determined by using a technique (including choice of spectrum line pair, optical adjustments, emulsion calibration method, photographic processing, and type of photographic emulsion, electrodes, and discharge) which is acceptable to the spectrographer and the manufacturer of the equipment.

d. The standard or routine samples of known concentration used to calibrate the analytical working curve and to determine the value,  $\delta$ , shall be acceptable only if the equipment manufacturer and spectrographer agree that the sample material used is representative of a satisfactorily homogeneous specimen. The degree of homogeneity of routine samples should be known prior to the installation of the equipment.

4. The day-to-day accuracy of the excitation equipment used in conjunction with a spectrograph as specified in I and a densitometer or microphotometer as specified in II shall be such that any correction factors, as determined by checking the accepted standard samples with the established technique, shall not shift the original analytical working curve beyond the values in per cent concentration of the element in the samples as given in column 4 of Table I<sup>1</sup>, over a test period of 90 days, provided:

a. The spectrographer has not varied his technique in obtaining the results in question.

b. The spectrographer has maintained all parts of the equipment as specified by the manufacturer of the equipment.

#### IV. AUXILIARY EQUIPMENT

1. The maximum variation in input voltage permitted for any unit of the installation, while achieving the ultimate precision and accuracy attainable from the unit, shall so be specified by the manufacturer of the equipment in order that a proper decision may be made concerning auxiliary input voltage control.

2. Any other auxiliary equipment or information required to obtain the performance as described in these requirements shall so be specified by the manufacturer of the equipment.

#### V. GENERAL REMARKS

These requirements are subject to change for reasons as follows by a majority vote of any group adopting them:

a. Advancements in the field of emission spectrography.

b. Unfair disadvantages to either spectrographer or equipment manufacturer.

#### FINAL DISCUSSION

If these requirements are found to be too rigid or too lax, the conditions may be changed by the majority opinion of any group or society considering such requirements. Nevertheless, the minimum capabilities of the equipment at least will be expressed in concrete terminology.

It is admitted that the requirements may emphasize the viewpoint of a spectrographer. If any incompleteness or unintentional unfairness has been noted, the suggestions and criticism of all interested spectroscopists, equipment manufacturers, and other spectrographers will be most welcome and appreciated. If the reader has been convinced of the need for such requirements, the paper has served its purpose.

<sup>1</sup> In this case column 1 of Table I represents the concentration values on the original working analytical curve. The values given in column 4 are equivalent to twice the values as given in column 2. The maximum working curve shifts for the low range have been left open for discussion as indicated by the question marks.

Table I. Precision and Day-to-Day Accuracy

Mean % Concentration of Element in Sample, $c$	Average Deviation from Mean in % Concentration of Element in Sample, $\delta$	Per Cent Average Deviation	Maximum Working Curve Shift in % Concentration of Element in Sample
Low Range			
0.0001	0.00005	50.0	?
0.001	0.00025	25.0	?
0.01	0.0012	12.0	?
0.05	0.0035	7.0	?
0.10	0.0057	5.7	?
Medium Range			
0.01	0.003	30.0	$\pm 0.006$
0.05	0.007	14.0	0.014
0.10	0.010	10.0	0.020
0.50	0.024	4.8	0.048
1.00	0.035	3.5	0.070
2.00	0.050	2.5	0.100
3.00	0.063	2.1	0.126
4.00	0.073	1.83	0.146
5.00	0.082	1.64	0.164



## ACKNOWLEDGMENT

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THE opinions expressed in this article are those of the author and not of the Ordnance Department.

# Qualitative Spectrographic Analysis

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A system of qualitative analysis for the metallic elements using the spectrograph as a tool, is described and its advantages and limitations are indicated. Allowance is made for differences in sensitivity by comparing the unknown spectrum with standard spectra which permit a semiquantitative estimation of each element found.

THE usefulness of the spectrograph as a tool for qualitative chemical analysis has long been recognized. During the last 15 years, however, the emphasis has been on quantitative analysis, in which field much research has been carried out, resulting in widespread use of the spectrograph in the analytical laboratory. Meanwhile, qualitative analysis continues as a useful but less publicized function of the spectrograph. This paper describes a system of qualitative analysis for the metallic elements and indicates its advantages and its limitations.

Identification of elements in spectrographic analysis is very positive even when dealing with very low concentrations. The sensitivity is generally excellent and superior to other methods. The very sensitivity of spectrographic analysis, however, is often its most disconcerting feature. When the analyst reports the presence of fifteen to twenty elements in a sample that was suspected of containing about five, the report is confusing unless the identification is accompanied by a statement of the approximate relative concentrations of the elements found. The more semiquantitative a qualitative analysis can be made, the more useful it is.

The requirements of a qualitative analysis may best be indicated by listing three of the principal applications:

1. As a preliminary to quantitative analysis, to indicate what elements should be determined and what separations may be required to avoid interferences.

2. As an aid to qualitative x-ray diffraction analysis to limit the search for an x-ray pattern match to compounds containing the principal elements found.

3. To detect the presence of beneficial or deleterious trace elements in raw materials and finished products.

In the first two of these and in other applications, a semiquantitative analysis is much more valuable than simple identification of the presence or absence of constituents.

In the early stages of the practice of qualitative analysis in this laboratory, the analytical report of the elements found was accompanied by a visual estimate of the intensities of the spectral lines, using the following arbitrary designations: vs, very strong; s, strong; m, moderate; w, weak; f, faint; vf, very faint; and xf, extremely faint.

In interpreting such an analysis, allowance must be made for the relative sensitivities of detection of the various elements—for ex-

ample, phosphorus reported as weak would be present to a much higher concentration than magnesium also reported as weak. Thus, the person unacquainted with the relative sensi-

Table I. Range of Spectrum in Which Elements May Be Detected with Greater Sensitivity

Range 1 (7000-3200 Å.)	Range 2 (3400-2370 Å.)	Equal Sensitivity in Range 1 or 2
Ba, Ca, Cr, Cs, Li, Na, Rb, Sr	Ag, Al, As, Au, B, Be, Bi, Cb, Cd <sup>a</sup> , Ce, Co, Cu, Er, Fe, Ga, Ge, Hg, In, Ir, La, Mg, Mn, Mo, Nd, Ni, P, Pb, Pd, Pt, Sb, Si, Sn, Ta, Te, Th, Ti, U, V, W, Y, Zn, Zr	K <sup>b</sup> , Rh, Tl

<sup>a</sup> For greatest sensitivity in detection of Cd, a line at 2288.018 Å. beyond Range 2 must be used.

<sup>b</sup> For greatest sensitivity in detection of K, a red-sensitive plate such as Eastman Spectroscopic Plate 1-L must be used to record the 7664.9 and 7698.9 Å. lines.

Table II. Master Standards for Identification

Standard No.	Element and Percentage in Zinc Matrix			
	10.0%	1.0%	0.01%	0.0001%
1	...	...	Ca	Al, Cd, Cu, Pb, Ag, Bi, Mg, Sn, Mn
2	...	...	As	Co, Cr, Ga, Sb, Be, Ge, Ni, Ti, V
3	...	...	P, Tl	In, Ir, Pd, Pt, Au, Mo
4	...	...	Ce, La, Ta, Rh, W	Er, Hg, Th, Y, B
5	Na, K, Cs, Rb	SrBa, Li	Te ...	...
6	...	U, Nd	Cb ...	Si, Fe ...

Table III. Most Sensitive Lines and Percentage Limits of Detection

Element	Lines, Å.	Limit of Detection, %	Use Range	Element	Lines, Å.	Limit of Detection, %	Use Range
Ag	3280.683	0.0001	II	Mg	2852.129	0.0001	II
Al	3092.713-3082.155	0.001	II	Mn	2794.817-2576.104	0.0001	II
As	2780.197-2860.452	0.01	II	Mo	3132.594	0.0001	II
Au	2675.95-2427.95	0.0001	II	Na	5895.923-5889.953	0.0001	I
B	2497.735	0.0001	II	Nd	3328.270-3133.603	1.0	II
Ba	4934.086-6141.716	0.01	I	Ni	3050.819-3002.491	0.0001	II
Be	3131.072-3130.416	0.0001	II <sup>a</sup>	P	2535.65	0.01	II
Bi	3067.716	0.0001	II	Pb	2833.069-2614.178	0.0001	II
Ca	4226.728-3933.666	0.001	I	Pd	3242.703	0.01	II
Cb	3194.977	0.001	II	Pt	3064.712-2997.967	0.001	II
Cd	3261.057	0.001	II <sup>b</sup>	Rb	6298.327	1.0	I
Ce	3201.714	0.01	II	Rh	3434.893-3396.85	0.001	I or II
Co	2424.930-2521.363	0.001	II	Sb	2598.062	0.001	II
Cr	4254.346-4274.803	0.0001 <sup>a</sup>	I	Si	2881.578-2516.123	0.0001	II
Cs	4593.177	1.0	I	Sn	2839.989	0.0001	II
Cu	3247.540-3273.962	0.0001	II	Sr	4607.331-4077.714	0.01	I
Er	3289.36	0.01	II	Ta	2714.674	0.01	II
Fe	3020.640-2979.352	0.0001	II	Te	2385.76	0.001	II
Ga	2943.637-2874.244	0.001	II	Th	2837.299	0.01	II
Ge	2651.178	0.0001	II	Ti	3372.800-3234.516	0.001	II
Hg	2536.519	0.001	II	Tl	5350.46-2767.87	0.01	I or II
In	3256.090-3039.356	0.001	II	U	2889.627	1.0	II
Ir	3220.780-2924.792	0.01	II	V	3183.982-2908.817	0.001	II
K	4044.140-3217.017	10.0	I or II <sup>c</sup>	W	2946.981	0.001	II
La	3337.488	0.1	II	Y	3242.280-3216.682	0.01	II
Li	6707.844	0.001	I	Zr	3391.975	0.001	II
					3345.020-3302.588	0.01	II <sup>d</sup>

<sup>a</sup> Most sensitive Be, 2348.6 in Range III.

<sup>b</sup> Most sensitive Cd, 2288.018 in Range III.

<sup>c</sup> Most sensitive K, 7664.9 and 7698.9 Å. A red-sensitive photographic plate such as Eastman spectroscopic 1-L must be used, which yields a sensitivity of 0.01% for potassium.

<sup>d</sup> Graphite base.



Table IV. Concentration of Elements Which May Interfere with Identification of Traces of Element Sought

Concentration Expressed in Per Cent in Zinc Matrix

Element Sought	Wave Length	Ag	Al	As	Au	B	Ba	Be	Bi	Ca	Cb	Cd	Ce	Co	Cr	Cs	Cu	Er	Fe	Ga	Ge	Hg	In	Ir	K	La	Li
Ag	3280.683	..	..	..	..	..	..	..	..	..	..	..	10.0	..	1.0	..	..	..	..	..	..	..	..	..	..	..	..
Al	3382.891	..	..	..	..	..	..	..	..	..	..	..	10.0	..	1.0	..	..	1.0	..	..	..	..	..	..	..	..	..
As	3092.713	..	..	..	..	..	..	..	..	..	..	..	10.0	..	10.0	..	..	..	..	..	..	..	..	10.0	10.0	1.0	..
As	3082.155	..	..	..	..	..	..	..	1.0	..	0.1	..	10.0	..	10.0	..	..	..	..	..	..	..	..	..	..	..	..
Au	2780.196	..	..	..	..	..	..	..	..	..	0.1	..	10.0	1.0	..	..	..	..	10.0	..	..	..	..	..	..	..	..
Au	2860.452	..	..	..	..	..	..	..	..	..	0.1	..	10.0	..	..	..	..	..	10.0	..	..	..	..	..	..	..	..
B	2675.95	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	10.0	..	..	..	..	..	..	..	..
B	2427.95	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	10.0	..	..	..	..	..	..	..	..
B	2497.733	..	..	..	..	..	..	..	..	..	..	..	..	1.0	..	..	..	..	10.0	..	..	..	..	..	..	..	..
Ba	2496.778	..	..	..	..	..	..	..	..	..	1.0	..	1.0	..	..	..	..	..	..	..	..	1.0	..	..	..	..	10.0
Be	2634.783	..	..	..	..	..	..	..	..	..	0.01	..	1.0	..	..	..	..	1.0	..	..	..	..	..	..	..	..	..
Be	3130.416	..	..	..	..	..	..	..	..	..	0.01	..	1.0	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Bi	3131.072	..	..	..	..	..	..	..	..	..	0.01	..	1.0	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Ca	3067.716	..	..	..	..	..	..	..	..	..	..	..	10.0	0.1	10.0	..	..	..	1.0	..	..	..	..	10.0	10.0	10.0	10.0
Ca	3179.332	..	..	..	..	..	..	..	..	..	..	..	10.0	..	..	..	..	..	10.0	..	..	..	..	10.0	10.0	10.0	10.0
Cb	3158.869	..	..	..	..	..	..	..	..	..	..	..	10.0	0.1	..	..	..	..	10.0	..	..	..	..	10.0	10.0	10.0	10.0
Cd	3194.977	..	..	..	..	..	..	..	..	..	..	..	0.1	..	..	..	..	..	10.0	..	..	..	..	10.0	10.0	10.0	10.0
Cd	3094.183	..	..	..	..	..	..	..	..	..	..	..	1.0	0.1	..	..	..	..	10.0	..	..	..	..	10.0	10.0	10.0	10.0
Ce	3261.057	..	..	..	..	..	..	..	..	..	..	..	1.0	..	..	..	..	..	10.0	..	..	..	..	10.0	10.0	10.0	10.0
Ce	3201.714	..	..	..	..	..	..	..	..	..	..	..	1.0	..	..	..	..	..	10.0	..	..	..	..	10.0	10.0	10.0	10.0
Co	3194.825	..	..	..	..	..	..	..	10.0	..	0.01	..	10.0	..	..	..	..	..	10.0	..	..	..	..	1.0	1.0	10.0	10.0
Co	3405.120	..	..	..	..	..	..	..	..	..	1.0	..	10.0	..	..	..	..	..	10.0	..	..	..	..	1.0	1.0	10.0	10.0
Cr	2521.363	..	..	..	..	..	..	..	..	..	..	..	10.0	10.0	..	..	10.0	..	1.0	..	..	1.0	..	1.0	10.0	10.0	10.0
Cr	2424.930	..	..	..	..	..	..	..	..	..	..	..	10.0	10.0	..	..	..	..	10.0	..	..	..	..	..	..	..	10.0
Cr	3021.558	..	..	..	..	..	..	..	..	..	..	..	10.0	10.0	..	..	10.0	..	1.0	..	..	..	..	..	..	..	10.0
Cu	2835.633	..	..	..	..	..	..	..	..	..	..	..	10.0	10.0	..	..	..	..	10.0	..	..	..	..	..	..	..	10.0
Cu	3247.540	..	..	..	..	..	..	..	..	..	..	..	10.0	10.0	..	..	..	..	1.0	..	..	..	..	..	..	..	10.0
Cu	3273.962	..	..	..	..	..	..	..	..	..	..	..	10.0	10.0	..	..	..	..	1.0	..	..	..	..	..	..	..	10.0
Er	3289.36	..	..	..	..	..	..	..	..	..	10.0	..	10.0	..	..	..	..	..	10.0	..	..	..	..	..	..	..	10.0
Er	2891.387	..	..	..	..	..	..	..	..	..	..	..	10.0	10.0	..	..	..	..	10.0	..	..	..	..	..	..	..	10.0
Fe	3020.640	..	..	..	..	..	..	..	..	..	10.0	..	10.0	10.0	1.0	..	..	..	10.0	..	..	..	..	..	..	..	10.0
Fe	3021.073	..	..	..	..	..	..	..	..	..	10.0	..	10.0	10.0	0.1	..	..	..	10.0	..	..	..	..	..	..	..	10.0
Fe	2973.237	..	..	..	..	..	..	..	..	..	10.0	..	10.0	10.0	10.0	..	..	..	10.0	..	..	..	..	..	..	..	10.0
Ga	2973.134	..	..	..	..	..	..	..	..	..	..	..	10.0	10.0	..	..	..	..	10.0	..	..	..	..	..	..	..	10.0
Ga	2943.637	..	..	..	..	..	..	..	..	..	..	..	10.0	10.0	..	..	..	..	10.0	..	..	..	..	..	..	..	10.0
Ga	2874.244	..	..	..	..	..	..	..	..	..	..	..	10.0	10.0	..	..	..	..	10.0	..	..	..	..	..	..	..	10.0
Ge	2651.178	..	..	..	..	..	..	0.1	..	..	1.0	..	1.0	..	..	..	..	..	0.01	..	..	..	..	..	..	..	10.0
Hg	3039.064	..	..	..	..	..	..	..	..	..	1.0	..	10.0	1.0	10.0	..	..	..	10.0	..	..	..	..	..	..	..	10.0
Hg	2636.519	..	..	..	..	..	..	..	..	..	..	..	10.0	1.0	10.0	..	..	..	10.0	..	..	..	..	..	..	..	10.0
In	3256.090	..	..	..	..	..	..	..	..	..	..	..	10.0	1.0	10.0	..	..	..	10.0	..	..	..	..	..	..	..	10.0
Ir	3039.356	..	..	..	..	..	..	..	..	..	..	..	10.0	1.0	10.0	..	..	..	10.0	..	..	..	..	..	..	..	10.0
Ir	3220.780	..	..	..	..	..	..	..	..	..	..	..	10.0	1.0	10.0	..	..	..	10.0	..	..	..	..	..	..	..	10.0
K	2924.792	..	..	..	..	..	..	..	..	..	..	..	10.0	1.0	10.0	..	..	..	10.0	..	..	..	..	..	..	..	10.0
K	3217.017	..	..	..	..	..	..	..	..	..	..	..	10.0	1.0	10.0	..	..	..	10.0	..	..	..	..	..	..	..	10.0
La	3337.488	..	..	..	..	..	..	..	..	..	..	..	1.0	1.0	1.0	..	..	..	1.0	..	..	..	..	..	..	..	10.0
La	3245.120	..	..	..	..	..	..	..	..	..	..	..	1.0	1.0	1.0	..	..	..	1.0	..	..	..	..	..	..	..	10.0
Li	3232.61	..	..	..	..	..	..	..	..	..	1.0	..	1.0	1.0	1.0	..	..	..	1.0	..	..	..	..	..	..	..	10.0
Li	2741.31	..	..	..	..	..	..	..	..	..	1.0	..	1.0	1.0	1.0	..	..	..	1.0	..	..	..	..	..	..	..	10.0
Mg	2852.129	..	..	..	..	..	..	..	..	..	10.0	..	1.0	1.0	1.0	..	..	..	1.0	..	..	..	..	..	..	..	10.0
Mn	2794.817	..	..	..	..	..	..	..	..	..	10.0	..	1.0	1.0	1.0	..	..	..	1.0	..	..	..	..	..	..	..	10.0
Mn	2798.271	..	..	..	..	..	..	..	..	..	..	..	1.0	1.0	1.0	..	..	..	1.0	..	..	..	..	..	..	..	10.0
Mo	2605.688	..	..	..	..	..	..	..	..	..	..	..	1.0	1.0	1.0	..	..	..	1.0	..	..	..	..	..	..	..	10.0
Mo	2593.729	..	..	..	..	..	..	..	..	..	..	..	1.0	1.0	1.0	..	..	..	1.0	..	..	..	..	..	..	..	10.0
Mo	2576.104	..	..	..	..	..	..	0.01	..	10.0	10.0	10.0	1.0	1.0	1.0	..	..	..	1.0	..	..	..	..	..	..	..	10.0
Na	3132.594	..	..	..	..	..	..	..	..	10.0	..	..	1.0	1.0	1.0	..	..	..	1.0	..	..	..	..	..	..	..	10.0
Na	3170.347	..	..	..	..	..	..	..	..	10.0	..	..	1.0	1.0	1.0	..	..	..	1.0	..	..	..	..	..	..	..	10.0
Nd	2852.828	..	..	..	..	..	..	..	..	..	..	..	1.0	1.0	1.0	..	..	..	1.0	..	..	..	..	..	..	..	10.0
Nd	2853.031	..	..	..	..	..	..	..	..	..	..	..	1.0	1.0	1.0	..	..	..	1.0	..	..	..	..	..	..	..	10.0
Ni	3328.270	..	..	..	..	..	..	..	..	..	..	..	1.0	1.0	1.0	..	..	..	1.0	..	..	..	..	..	..	..	10.0
Ni	3133.603	..	..	..	..	..	..	..	..	..	..	..	1.0	1.0	1.0	..	..	..	1.0	..	..	..	..	..	..	..	10.0
Ni	3050.819	..	..	..	..	..	..	..	..	..	..	..	1.0	1.0	1.0	..	..	..	1.0	..	..	..	..	..	..	..	10.0
P	3002.491	..	..	..	..	..	..	..	..	..	..	..	1.0	1.0	1.0	..	..	..	1.0	..	..	..	..	..	..	..	10.0
Pb	2535.65	..	..	..	..	..	..	..	..	..	..	..	1.0	1.0	1.0	..	..	..	1.0	..	..	..	..	..	..	..	10.0
Pb	2833.069	..	..	..	..	..	..	..	..	..	..	..	1.0	1.0	1.0	..	..	..	1.0	..							



[illegible]

(Continued on page 878)



(Continued from page 677)

Element Sought	Wave Length	Na	Mg	Mn	Mo	Na	Nd	Ni	P	Pb	Pd	Pt	Rb	Rh	Sb	Si	Sn	Sr	Ta	Te	Th	Ti	Tl	U	V	W	Y	Zn	Zr
Na	3852.828	1.0			0.1							1.0												10.0	0.1	10.0			0.1
Nd	2853.031	1.0			10.0			0.1																10.0	10.0	1.0			
Ni	3328.270						10.0																	10.0	10.0	1.0	0.01		
P	3133.603				10.0																			10.0	10.0	1.0			
Pb	3050.819		1.0		10.0																			10.0	10.0	1.0			
Pd	3002.491		10.0								0.1													10.0	10.0	1.0			
Pt	2535.65						10.0																	10.0	10.0	1.0			
Rh	2833.069				1.0		10.0	0.1																10.0	10.0	1.0			0.1
Sb	3242.703				1.0		10.0																	10.0	10.0	1.0			1.0
Sn	3404.580				1.0		10.0	0.01																10.0	10.0	1.0			0.1
Sr	3064.712				10.0		10.0	10.0																10.0	10.0	1.0			1.0
Ta	2997.967				3396.85		10.0																	10.0	10.0	1.0			1.0
Te	3323.092						10.0																	10.0	10.0	1.0			1.0
Th	2598.062						10.0																	10.0	10.0	1.0			1.0
Ti	2839.989						1.0																	10.0	10.0	1.0			1.0
Tl	2863.327						1.0	0.01																10.0	10.0	1.0			1.0
U	3380.711						1.0																	10.0	10.0	1.0			1.0
V	2714.674																							10.0	10.0	1.0			1.0
W	2647.472																							10.0	10.0	1.0			1.0
Y	2385.76																							10.0	10.0	1.0			1.0
Zr	2383.26																							10.0	10.0	1.0			1.0
	2837.299																							10.0	10.0	1.0			1.0
	2832.319																							10.0	10.0	1.0			1.0
	3234.516																							10.0	10.0	1.0			1.0
	3383.761																							10.0	10.0	1.0			1.0
	3372.800																							10.0	10.0	1.0			1.0
	3199.915																							10.0	10.0	1.0			1.0
	2767.87																							10.0	10.0	1.0			1.0
	2889.627																							10.0	10.0	1.0			1.0
	2865.679																							10.0	10.0	1.0			1.0
	3183.982																							10.0	10.0	1.0			1.0
	3093.108																							10.0	10.0	1.0			1.0
	2946.981																							10.0	10.0	1.0			1.0
	2944.395																							10.0	10.0	1.0			1.0
	3242.280																							10.0	10.0	1.0			1.0
	3216.682																							10.0	10.0	1.0			1.0
	3391.975																							10.0	10.0	1.0			1.0

tivities would be misled by an analytical report based only on line intensity.

As a result of this weakness of the method, a system was devised to allow for differences in sensitivity by comparing the unknown spectrum with standard spectra which permit a semiquantitative estimation of each element found. The details of the spectrography and interpretation are given in this paper.

SPECTROGRAPHY

Of the various means of exciting the spectra, the direct current arc with graphite electrodes is probably the best for general detection of metallic constituents from the standpoint of sensitivity, general applicability, and convenience. The electrodes are mounted vertically, the sample being placed in a crater drilled in the tip of the lower electrode. This means of excitation is used in nearly all cases of qualitative analysis in this laboratory. A large Littrow-type quartz spectrograph is used to record the spectra

One procedure is to place approximately 0.1 gram of the unknown in a crater drilled in the tip of a graphite electrode. With this electrode as positive pole and a counterelectrode of graphite, a 10-ampere direct current arc is struck to excite the spectrum. The exposure is continued until all the sample appears to have been volatilized, 5 to 15 minutes, depending upon the nature of the sample. Under such conditions, the more volatile elements are vaporized in the initial stages and the more refractory elements fuse down to a bead at the bottom of the crater and are vaporized more slowly. Complete vaporization of the sample is necessary for a reliable analysis. In this paper this type of arc is referred to as the conventional direct current arc.

For several years this laboratory has been using a more rapid technique, the Hasler high streaming velocity arc (1, 2) which has been adapted to qualitative analysis.

The sample is put into the form of a fine powder and intimately mixed with carbon powder obtained by the charring of sucrose. (Graphite powder is not suitable for this purpose.) This mixture is packed into the crater of a specially formed graphite electrode and then volatilized in the direct current arc. Under these conditions the sample passes very rapidly into the arc flame, the gases developed by the burning sucrose carbon powder literally blowing the powder into the arc flame. Most samples are completely removed from the electrode in 0.5 minute. This type of arc seems to effect a uniform transfer of the sample from the electrode to the arc as compared to the fractional distillation taking place in the conventional arc.

The tip of a 0.78-cm. (0.3125-inch) diameter graphite electrode is drilled with a special cutter so as to form an annular hole 0.6 cm. (0.25 inch) in outside diameter, 0.3 cm. (0.125 inch) in inside diameter, and 0.78 cm. (0.3125 inch) deep. This leaves a center pole of 0.3 cm. (0.125 inch) diameter at the center of the crater. The unknown sample is dissolved in acid, preferably nitric, and the solution evaporated to dryness in a fused quartz dish, and heated gently over a Bunsen burner substantially to decompose the nitrates into oxides. If the sample is already in powder form, this solution step may be omitted. The powder is then ground in an agate mortar to a fineness equivalent to through 200-mesh and thoroughly mixed. The powder should not be screened because of the danger of contamination. Approximately equal volume portions of the powdered sample and sucrose carbon are intimately mixed and ground in an agate mortar and the annular hole electrode crater is filled with this mixture. A special metal funnel is used, fitting over the



electrode in such a manner that none of the powder is lost during transfer. With zinc-base samples, a standard quantity of 50 mg. of sample powder and 50 mg. of sucrose carbon is used. In all cases exposure time is standardized at one minute.

Spectra obtained from this type arc are much more free of background than the arc formerly used and the exposure time is only one tenth as long.

Comparative tests have been made between the conventional direct current arc and the high streaming velocity arc on a variety of samples. The relative concentrations of the elements in an unknown are indicated to be substantially the same by the two methods. The high streaming velocity arc method is somewhat more sensitive, since a few trace elements are usually detected which were not detected by the conventional arc method. The very large decrease in exposure time results in an important increase in speed, and saving in labor.

Most metals have their most sensitive lines in the ultraviolet portion of the spectrum. A few metals (barium, calcium, chromium, cesium, lithium, sodium, rubidium, and strontium) show greater sensitivity in the visible spectrum, however, and if the amounts of these elements are small their spectral lines may have to be sought also in the visible spectrum. With the Littrow spectrograph, this requires the taking of two plates. In this laboratory these two spectral regions are designated as Range 1 (or visible) extending from 7000 to 3200 Å., and Range 2 (or ultraviolet) extending from 3400 to 2370 Å. Table I shows the range of the spectrum in which the elements may be detected with greater sensitivity.

The data of Table I are based on experimental tests of sensitivity in a zinc matrix, using the direct current arc between graphite electrodes. With other matrices, other supporting electrodes, self-electrodes, other excitation conditions, or other photographic plates, the grouping would probably be different.

As a matter of routine practice the spectrum is usually photographed in Range 2 only. Evidence for all of the elements listed in Table I is sought. If some elements not detected in Range 2 and having a greater sensitivity in Range 1 are of especial interest in the particular sample being analyzed, the spectrum will be rephotographed in Range 1. In some cases the spectra will be photographed in both ranges at the outset.

#### IDENTIFICATION

To facilitate spectral line identification, a comparison plate is used upon which are recorded the spectra of a group of master standards containing enough of the various elements in a zinc matrix to give several of the strongest lines of each. The compositions of each are listed in Table II.

In routine practice, no attempt is made to identify every line in the spectrum. Rather, a search is made only for each of 53 elements by reference to the master standard plates. Of the elements not specifically sought, eleven are gases, five are non-metals not detectable in the direct current arc, and twenty are rare metals.

The most sensitive lines under the conditions of spectrography specified in this paper and the approximate limits of detection are listed in Table III. No attempt is made to indicate the sensitivity more precisely than in order of magnitude.

Obvious precautions must be observed in identification. The more lines of a particular element which can be observed, the more certain is its identification. If only the most persistent line shows up faintly, the possibility that that line is a faint line in the spectrum of another constituent or from an electrode impurity must be considered.

A survey has been made of possible line interferences. These interferences may be due to the coincidence of a line of the interfering element with the persistent line of the element sought or to the proximity of a strong line of an interfering element. Table IV shows the concentration of interfering elements which may interfere with the identification of traces of elements sought. The data are derived from zinc-base standards and the percentages expressed in terms of the zinc matrix. The use of the table may be illustrated by an example. Suppose the only line of barium detectable in a sample is the 2634.783 line. If the sample contains 1% of columbium, or 10% of copper, molybdenum, or neodymium, the indicated presence of barium would be open to question.

In most cases, identification can be established by the detection of several lines of each element. If, however, only the most persistent line (or lines) of an element are found, then the possibilities of interference given in Table IV must be considered.

#### SEMIQUANTITATIVE EVALUATION

Each element found in the unknown is graded by comparison with the spectra of a series of standard samples made up in a zinc base. Since the standards are in a zinc base, their use is strictly valid only when analyzing zinc-base materials, but it has been the experience in this laboratory that these standards give useful results with other than zinc-base materials—results which are more valid than if no compensation was made for differences in spectral sensitivity of the various constituents of an unknown.

The standards are made as follows: Nine grams of zinc and 1 gram of the element are dissolved in nitric acid. (If the element cannot be dissolved in nitric acid, a small quantity of some other solvent is used to effect a solution, following which the solution of zinc in nitric acid is added.) The resulting solution, containing 90% zinc and 10% element, is converted to a well-mixed oxide powder in the manner described above for preparing unknown samples. In some cases it is not possible to achieve a clear solution, but this does not matter since the solution is evaporated to dryness and the powder is well ground and mixed. This constitutes the 10% standard. A one-tenth aliquot of this weight of powder is then dissolved in nitric acid, together with 9 grams of zinc. From this solution the second standard is prepared, equivalent to 1%. This process is continued until standards are available for 10, 1, 0.1, 0.01, 0.001, and 0.0001%. This is carried out for the 53 elements listed in Table III. In the case of the standards for the grading of zinc, a base of graphite powder is used.

The spectra of the six standards for each of the elements are photographed and kept on file as standard grading plates.

Table V. Reporting Designations Based on Relative Concentrations

Standard, %	Reporting Designation
>10.0	vs
10.0	s
1.0	m
0.1	w
0.01	f
0.001	vf
0.0001	xf

Table VI. Comparison between Qualitative and Quantitative Analyses

Sample A			Sample B			Sample C			Sample D			Sample E		
1 <sup>a</sup>	2 <sup>b</sup>	3 <sup>c</sup>	1 <sup>a</sup>	2 <sup>b</sup>	3 <sup>c</sup>	1 <sup>a</sup>	2 <sup>b</sup>	3 <sup>c</sup>	1 <sup>a</sup>	2 <sup>b</sup>	3 <sup>c</sup>	1 <sup>a</sup>	2 <sup>b</sup>	3 <sup>c</sup>
Zn	vs	..	Zn	vs	..	Cu	vs	87.8	Cu	vs	55.1	Fe	m-s	40.0
Pb	m	0.5	Ni	w	0.045	Al	w-m	8.8	Zn	m-s	42.0	Ti	m-s	8.6
Cd	w	0.17	Mg	f	0.08	Fe	w-m	2.2	Al	w-m	0.83	Si	m-s	4.4
Sn	f	0.006	Pb	f	0.055	Ni	w	..	Fe	w-m	0.83	Mg	w-m	3.4
In	f	0.005	Sn	f	0.027	Zn	f-w	<0.1	Mn	w-m	0.66	Ca	w-m	5.9
Fe	f	0.04	Fe	f	0.04	Ag	f-w	..	Pb	w	0.13	Al	w	1.2
Cu	f	0.023	Cu	f	0.025	Mn	f	..	Sn	f-w	0.18	Mn	f-w	0.35
Ag	vf	..	Cd	vf	0.002	Pb	vf-f	0.037	Ni	f	..	V	vf-f	..
Sb	vf	..	Mn	vf	0.006	Sn	vf-f	0.024	Ag	vf-f	..	Ga	vf	..
Ni	vf	0.0005	Mo	vf	..	Cr	vf	..	Cr	vf-f	..	Cu	xf-vf	..
			Bi	xf	0.0005	Be	xf-vf	..	Sb	xf-vf	..			

<sup>a</sup> Elements detected.

<sup>b</sup> Qualitative grading of relative concentration.

<sup>c</sup> Quantitative determination where available.



Table VII. Comparison of Moving Plate Method and High Streaming Velocity Arc

Sample	Found in M.P.M. but Not in H.S.V.A.M.	Found in H.S.V.A.M. but Not in M.P.M.
A	None	None
B	Bi, Be, Ga, Tl, P, Zr, Sr	None
C	Ga, As, Ge, Sb, P, Sr, Co	None
D	Ti	Zn
E	Ni, Cd	None
F	None	None

The spectrum of an unknown is first examined to determine what elements are present by reference to the master standard plates. The plate is then compared visually with the standard grading plates for the elements found, and an estimate is made of the standard to which each element intensity in the unknown most nearly approximates.

Each standard has been assigned an arbitrary grading designation for report purposes, as shown in Table V. An element graded as being nearer the 0.01% standard than to any other standard could be reported as approximately 0.01% or as f. An element judged to be about midway between 0.01 and 0.1% could be reported as approximately 0.05 or as f-w. It is the general practice in this laboratory to report the symbol rather than the approximate percentage. To report an actual figure in per cent, even though modified by the word "approximately", may be misinterpreted by some user of the results as an exact quantitative result.

There are several known sources of error. An effort is made to maintain uniform excitation conditions, plate sensitivity, and plate processing conditions, but these conditions are variable. The photographic errors are particularly serious. Differences in the major constituents of the sample introduce variations in the intensity of the spectra, which cannot be completely controlled or compensated for by any practical methods.

The analytical results on five samples selected at random from samples upon which both qualitative and quantitative analyses have been made are listed in Table VI. Two zinc-base, two copper-base, and one iron-base samples are included. These results indicate the degree of reliability to be expected from the semiquantitative indications given by this system of qualitative analysis.

The average time required for the identification and grading of a qualitative plate is 20 minutes.

#### MOVING PLATE METHOD

Occasional use is made of another technique designated as the moving plate method. No claim is made for originality in using this technique but the results obtained thereby in qualitative analysis are worthy of being recorded.

Using simple counter-type graphite electrodes, the sample is volatilized with the direct current arc and the spectrum recorded in successive increments of exposure over the entire 5- to 15-minute period which may be required to volatilize the entire sample. The author's practice is to photograph a spectrum 2 mm. wide for each 30 seconds of exposure, moving the plate 2 mm. vertically every 30 seconds during the total arcing period.

This technique results in a series of spectra showing the elements being volatilized during each increment of the arcing period. This sometimes gives greater sensitivity in detecting some elements, particularly those elements which volatilize over a short period at some portion of the arcing period, probably because the increment of spectrum exposure recorded at the time the particular element is being volatilized is not overexposed with general background.

The results shown in Table VII indicate that the moving plate method is more likely to detect all the elements present than the high streaming velocity arc method.

The additional elements found, however, are trace elements

giving very faint spectra. The moving plate method is much more time-consuming and is not used as a matter of routine unless circumstances indicate the need for the very best sensitivity and the most complete analysis.

In grading a plate, the practice is to search each spectrum for each of the 53 elements. The intensity grading, for each element found, is made on the increment of exposure showing this element most strongly, using the same standard grading plates as those used for the high streaming velocity arc. No justification for the validity of relative concentration gradings made in this manner can be made except to say that the gradings are generally in fair agreement with those made by the high streaming velocity arc method.

#### ACKNOWLEDGMENT

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## Alundum Gas Diffusers

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A VERY convenient and inexpensive gas diffuser for laboratory use can be made by sealing ordinary Pyrex glass tubing to Alundum extraction thimbles. Despite the fact that Alundum has about twice the expansion coefficient of Pyrex, satisfactory seals can be made between the two materials as

shown in Figure 1. The large sizes are made by blowing an enlargement in the tubing of desired size and butt-sealing this to the thimble. The smallest size is most conveniently made by lap-sealing the glass tubing to the thimble. The resistance of this type of diffuser is indicated by the data in Table I, showing typical pressure drops across the various size thimbles when passing 1000 ml. of air per minute through water. The Alundum thimbles are available from the Norton Company, Worcester, Mass., for about 50 cents each. The Norton Company recommends Alundum diffusers for use in acid but not in alkaline solutions.

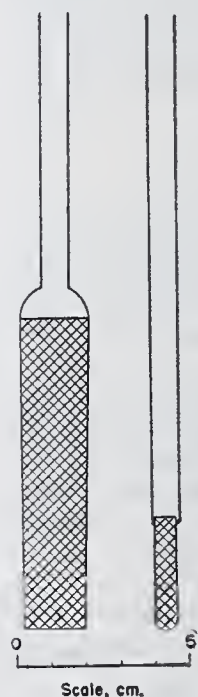


Figure 1

#### ACKNOWLEDGMENT

The authors acknowledge the assistance of L. A. Bedwell, who did the necessary glass blowing, and the permission of Westvaco Chlorine Products Corporation to publish this note.

Table I. Pressure Drop across Alundum Diffusers

(Air rate = 1000 ml. per minute)		
Dimensions, Mm.	Description	Pressure, Mm. of Hg
19 × 90	No. 6839, RA98	82
16 × 70	No. 11702, RA98	92
15 × 90	No. 7338, RA98	107
6 × 32	No. 8133, RA98, sanded	130
6 × 32	No. 8133, RA98	152



# Determination of Noncondensables in Gas

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An apparatus is described for the quantitative determination of small percentages of noncondensable in an easily condensed gas. The apparatus should be useful for analyses of many gaseous systems, especially in the case of gases for which no chemical absorbents are available. The vapor pressure and liquid density of the condensable gas must be known. The method has been applied to the determination of noncondensable in commercial difluorodichloromethane, Freon-12.

THE need has arisen in this laboratory in connection with research work on refrigeration for a method of quantitative determination of noncondensable in the presence of large amounts of easily condensed gas. Routine determinations were required for such analyses of dichlorodifluoromethane, boiling point  $-30^{\circ}\text{C}$ ., when the noncondensable was in the range of 0.001 to 3%. Atmospheric gases constituted the noncondensable.

A method developed by the Kinetic Chemicals Corporation (1) was available for the determination of nonabsorbable in kerosene, which in this case is nearly the same as noncondensable. Their method involves the absorption of the condensable gas in kerosene which has been freshly boiled and then saturated with air. However, the method was not satisfactory for the author's purposes, for several reasons. First, a rather large correction (1.6%) must be applied to compensate for air driven out of the kerosene by the dichlorodifluoromethane. Such a correction leads to inaccuracies in the low range of nonabsorbables. Furthermore, a method was desired which measured actual noncondensable without the assumption that it was the same as nonabsorbable. The apparatus described here proved to be rapid in operation and to give reproducible results in the whole range of

concentration desired. A complete analysis requires from 10 to 15 minutes, exclusive of calculation.

## PRINCIPLE OF THE METHOD

The gas is liquefied by cooling in a graduated tube. The amount of noncondensable is observed and corrected for the presence of condensable gas. The volume of condensate is observed, and converted to gas volume through the known densities of the two phases. From the volumes of condensable and noncondensable, the percentage of noncondensable is calculated to any desired basis.

## APPARATUS

The apparatus, shown in Figures 1 and 2, is of glass, with a rubber tube connection from A to a mercury leveling bulb. Tubes A, C, and J are of 7-mm. inside diameter. The capillary tubes have about 2-mm. bore. Bulbs F and E have volumes of about 0.5 and 1.5 ml., respectively. Tube G is graduated from point O in convenient increments of volume, depending on tube diameter. The first graduations below O are on the capillary, hence correspond to very small increments of volume. The next are on a 6-mm. tube, and the next on a 12-mm. tube, which is the main body of G. At the bottom of G is a short 4-mm. capillary, graduated at 2-mm. intervals to the mark above F. The total volume of G is 7.5 ml. The dimensions were calculated to give the maximum accuracy throughout the range of noncondensables expected. This calculation is based on the range of noncondensables to be covered, the relative densities of gas and liquid and the operating temperature. The apparatus was accurately calibrated by the use of mercury. The cold zone is an unsilvered 1-liter vacuum flask, filled with dimethoxytetraethylene glycol, and cooled with dry ice (2). A temperature of about  $-31^{\circ}\text{C}$ . can be maintained easily for long periods in this way. The flask can be lowered readily to allow the apparatus to warm.

A scale is placed behind C, for reading pressure, and the height from a reference point on the scale to the graduations of G is known. The graduations are calibrated for height, so that an observation of the mercury levels in C and G can be reduced to a pressure difference.

## MANIPULATION

By proper manipulation of the stopcocks, with the leveling bulb raised, the apparatus is filled completely with mercury up to the tip of tube K, and up tubes C and J to points about 20 cm. above the cocks. Connection is then made at K to the source of gas to be analyzed, and cocks B and H are turned to shut off the mercury in C and J, but to allow flow from K through to the mercury bulb. The mercury bulb is then lowered slowly, drawing in sample, until the mercury level is in bulb F. The vacuum flask, at the low temperature, is then raised to surround the apparatus, and condensation begins. During condensation, the mercury level is adjusted exactly to the mark at the bottom of F or E, B is shut off, and condensation continued till F or both bulbs are nearly filled with liquid. (This choice depends on the probable amount of noncondensable present, the larger sample when both bulbs are used permitting more accurate measurement if the percentage of noncondensable is small.) When the condensed liquid nearly reaches the mark at the top of F, cock H is turned off, and then turned to allow mercury to run down from J into the capillary to O, thus sealing the tube and driving all gas into the cold zone. (It is evident that the mercury height in J must be sufficient to overcome the pressure in the apparatus.)

The apparatus is then allowed to stand for several minutes, to allow the liquid to drain into F. With some practice, it is possible to stop the flow, so that this draining will fill the bulb almost exactly to the mark. Any excess can readily be estimated by the graduations on the capillary tube above F. The volume of condensable is thus measured as liquid, and its temperature estimated from that in the bath. The mercury bulb is then raised above B, and cock B turned to connect A, C, and D. The height of mercury in C, the top of the liquid meniscus in G, the mercury level in G, and the temperature of the bath are observed. By manipulation of the pressure, it is possible to adjust the liquid level to a favorable location for reading. From the barometric pressure and the mercury levels, corrected for a small head of liquid above the mercury in G, the total pressure on the gas is known. From the temperature and the thermodynamic tables of the liquid, the partial pressure of the liquid is known. Hence the partial pressure of the noncondensable is found, and this,

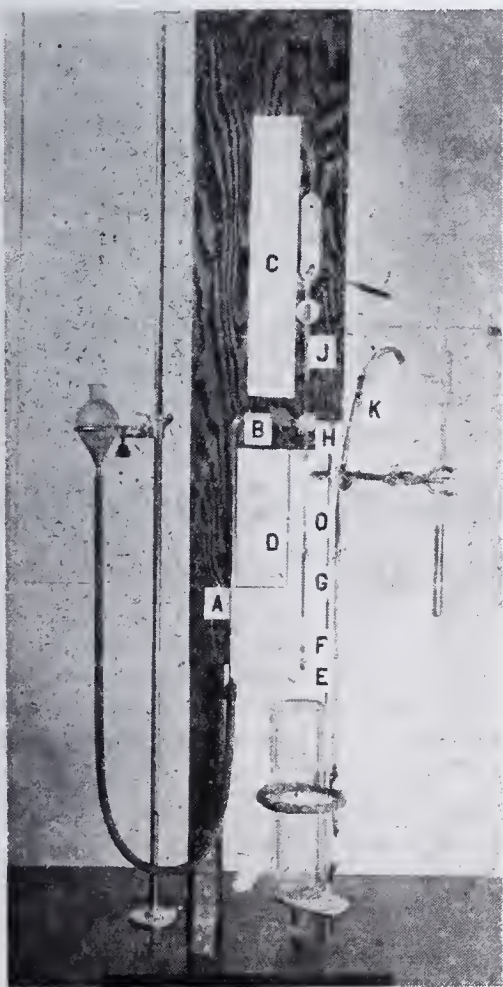


Figure 1



combined with its volume and temperature, leads to the amount of noncondensable, expressible in any desired way, since the amount of condensable is known. The amount of condensable in the vapor phase can be calculated, and added to the volume of condensate, but this correction is negligible.

To empty the apparatus, the mercury from *J* is slowly drawn through *H* into *G*, then *H* is opened, and the vacuum flask is removed. As the liquid boils, it escapes through *J*. A mercury trap at the top of *J* is desirable.

#### CHECK RESULTS

Some commercial material of high purity was analyzed by the method just described, giving reproducible results in the range 0.013 to 0.020% noncondensable. To this material was added air in measured proportions, after which the mixture was analyzed as described. The following results illustrate the reproducibility of the analyses as checked by the author in his work:

% Air Added	% Noncondensable Found
1.25	1.23
1.52	1.46
0.34	0.32
1.16	1.13
1.01	1.03
0.62	0.64

The discrepancy is probably due as much to the uncertainties in preparing the mixtures as to the analyses.

A source of error lies in the solubility of noncondensable in the condensate. This error, of course, depends on the system under investigation, and in some systems could easily be excessive. By keeping the partial pressure of the noncondensable low, the error can be minimized. In the measurements cited, the temperature of the condensate has been kept about 1° below its normal boiling point, and the pressure during condensation only a few inches greater than atmospheric. Hence the partial pressure of the gas is not more than 7.5 or 10 cm. (3 or 4 in.) of mercury. The temporary increase of partial pressure to about 25 or 30 cm. (10 or 12 inches) when the volume is observed probably results in little increase in the noncondensable dissolved. Condensation near the normal boiling point is probably most satisfactory, since with a small positive pressure in the apparatus the partial pressure of noncondensable is kept small. Furthermore, the vapor pressure of the condensable is frequently better known or more satisfactorily estimated near the normal boiling point. If the noncondensable data are to be used at temperatures other than that of the analyses, it is well to remember that "noncondensable" is a relative term and what is noncondensable at one temperature may be condensable at other temperatures.

If water is present in quantity, it must be removed before the analysis, or the tube will clog with frost. It is advisable to keep a slight positive pressure in the apparatus to avoid the possibility of leaks inward. There is some tendency for dichlorodifluoromethane to leak through ordinary stopcocks. The manipulations described were planned to reduce leakage to a minimum, by the use of moderate pressures, and by separating the gas and liquid from the stopcocks with mercury when possible. Repeated use of the apparatus tends to saturate the stopcock lubricant with gas, which may reduce the leakage. In the range of noncondensable considered here, small losses of condensable are much less important than leakage, either inward or outward, of noncondensable. The use of special stopcocks or of special grease

might reduce the leakage, but under the conditions used such refinements appear unnecessary.

Sampling of a gas mixture for analysis is just as important as the analysis, and frequently more difficult to do accurately. In this special case, uniform samples were available of sufficient quantity for an analysis (ca. 100 to 400 ml.). Frequently, however, gas of uniform composition might not be available, or might be available only in very small quantity. The sampling then could easily be a real problem, especially if two phases were present.

#### GENERAL APPLICATION

The apparatus should be useful with a wide variety of gases, especially those for which no chemical absorbents are available. Other temperatures of condensation can be used. Tube *C* can be connected at point *A* to cover lower pressures, and to provide for the simultaneous measurement of gas and liquid volumes. The addition of more liquid bulbs, or the change of relative volumes of the apparatus, could make the apparatus cover a different range of noncondensable. Provision can be made to withdraw and analyze the noncondensable. This has not been done. It is necessary to know the liquid density and vapor pressure of the condensable gas.

#### LITERATURE CITED

- (1) Kinetic Chemicals, Inc., Wilmington, Del., *Tech. Paper* (1931).
- (2) Wikoff, Cohen, and Grossman, *IND. ENG. CHEM., ANAL. ED.*, 12, 92 (1940).

## General Motors Spectrographic Conference

A series of spectrographic conferences has been initiated by the General Motors Corp., under the chairmanship of G. M. Rassweiler, Research Laboratories Division, to develop further the application of these analytical tools. Application of spectrochemical analysis has expanded so rapidly in connection with war production problems that 24 General Motors plants now have spectrographic installations in operation or on order.

Attended only by General Motors men, these conferences provide for frank and critical examination of methods now in use or contemplated. The fundamental physical and chemical aspects are discussed, together with closely associated problems of physics, chemistry, and metallurgy.

Five conferences have already been held, attended by from 40 to 60 men. Papers have been presented by R. E. Nusbaum and D. L. Frick of the Research Laboratories Division, S. F. Simpson of Chevrolet, W. N. Hatfield of Delco-Remy, R. W. Smith of AC Spark Plug, H. E. Grossman of Harrison Radiator, E. Osborne of Buick, F. D. Brookshire and W. R. O'Neill of Cadillac Motor, L. A. Danse of Standard Section, and R. B. Schenck of Buick. Further meetings are planned as well as such other activities as cooperative preparation of standards and extensive study of methods and equipment.

## Fifteen-Year Collective Index

Publication of the fifteen-year collective index to the ANALYTICAL EDITION of INDUSTRIAL AND ENGINEERING CHEMISTRY is now scheduled for February 15, 1945, and a domestic prepublication price of \$1.75 per copy has been set. Domestic price after publication will be \$2.25 per copy. Foreign, 10 cents additional.

This index, prepared by Charles L. Bernier, associate editor of *Chemical Abstracts*, will be a book of more than 100 pages, of the same size as regular issues of the ANALYTICAL EDITION. In preparing the index the style of *Chemical Abstracts* has been followed in general, with modifications to make it more adaptable to its special uses. All articles published from 1929 through 1944 are included. This index has been built from scratch, not made by combining yearly indexes. The indexing is uniform and harmonized throughout.

Orders, with check in payment, should be sent with the card inserted in this issue, to the AMERICAN CHEMICAL SOCIETY, 1155 Sixteenth St., N.W., Washington 6, D. C.

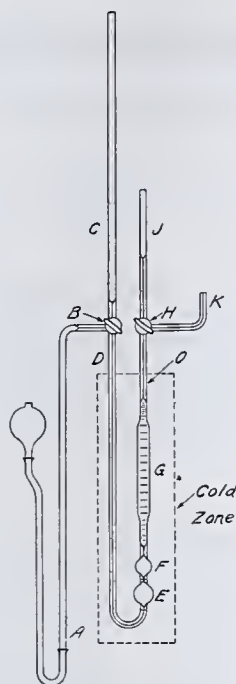


Figure 2



# Viscometric Chain Length of Wood Cellulose in Triton F Solution

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Use of aqueous dimethyldibenzylammonium hydroxide (Triton F) as a cellulose solvent for the determination of average chain length by the viscometric method, in dilute solution, is suggested. A suitable experimental technique is described in detail. Intrinsic viscosity data for a number of cotton and wood celluloses in Triton F are compared with the corresponding degree of polymerization values obtained viscometrically after nitration, and a linear relationship is established. The results are compared with cuprammonium viscosity values, as used in the wood cellulose industry.

FOR purposes of fundamental research on cellulose in its various forms (linters, wood celluloses, etc.) the need for a good cellulose solvent, giving solutions rather stable to light and air, is readily evident.

At present the only such solvent known appears to be an aqueous solution (about 35%) of the strong base dimethyldibenzylammonium hydroxide, commercially available in experimental quantities as Triton F (Rohm and Haas). The high solvent power of this basic solution has been conclusively shown by Clibbens and co-workers (3), who measured the solubility of cottons in the base at different temperatures and concentrations. They found that Triton F "permits complete dissolution of an unmodified cotton at 20° C.". This has been substantiated by other workers. Russell and co-workers (12, 13) have made a detailed study of the methods of dissolving cellulose in this solvent, with attention to time, temperature, concentration of the Triton base, and mode of stirring during solution. They found that at 25° C. there exists a narrow range of concentrations in which the dissolving power is maximum—namely,  $1.96 \pm 0.01 N$ . This point shifted with temperature, moving to lower concentrations at lower temperatures. For the solution of celluloses of the very highest viscosity, base concentrations of 2.1 to 2.25  $N$  gave better results. Brownsett and Clibbens (3) give the point of maximum dissolving power as 1.95  $N$  at 20° C., with a second maximum at about 2.5  $N$ . Once the cellulose solution has been formed, it is no longer necessary to maintain a concentration of base as high as that required to effect solution—for example, dilution of a 1% solution of unmodified cotton cellulose to one-half concentration is possible without precipitation. In general, precipitation does not start until the base concentration is reduced below 0.5  $N$ .

Two methods of efficiently dissolving cellulose samples in Triton F have been described. In one (12), the cellulose-solvent mixture is mechanically stirred in a large test tube with a heavy glass rod arranged to sweep out a circular path as close as possible to the test-tube wall. By this method, about 2 to 3 hours are required to form a 1% solution. In the second method (10), the cellulose and solvent are placed in an all-glass vial containing a glass plunger, and rolled for about 18 hours.

The stability of Triton F cellulose solutions to light and air has been established (13) by comparing the viscosities of the solutions when prepared under nitrogen with the viscosities obtained in the presence of air or oxygen. Such demonstrations of the stability of this alkaline solution of cellulose show a most remarkable contrast to the well-known behavior (1) of cuprammonium cellulose solutions. However, Triton F cellulose solutions have been shown (13) to be subject to a slow viscosity drop with time, the effect being accelerated by a rise in temperature, and furthermore being proportionately greater for higher concentrations of dissolved cellulose. This aging effect has not been satisfactorily explained.

It has been found (12, 13) that the specific viscosities of Triton F solutions of normal and degraded cottons, over a wide range of viscosities, vary in an approximately linear manner with the specific viscosities of cuprammonium solutions at the same cellulose concentration (0.5%). This led to the suggestion that Triton F should be substituted for cuprammonium reagent in commercial practice, in view of the greater stability of its solutions.

Further viscosity data provided in the work of Brownsett and Clibbens (4) show especially a unique feature of Triton F viscosity values. Comparison of the viscosities of various oxycelluloses measured in Triton F and cuprammonium reagent, before and after a weak alkaline treatment, reveals that the celluloses having alkali-sensitive linkages (14) are not fully hydrolyzed by cuprammonium reagent, whereas Triton F is a strong enough base to split completely all such points of latent degradation. In other words, viscosity measurements in cuprammonium give a value for apparent degradation, whereas in Triton F both apparent and latent degradation are measured.

All these interesting and advantageous properties of Triton F suggest its use as an ideal cellulose solvent for viscometric chain-length determinations, using the very dilute solutions required for such a purpose, rather than the relatively concentrated solutions heretofore reported in the literature. The present paper describes in some detail the experimental method developed through three years of constant use, and provides a mathematical constant for converting the measured viscosity values into terms of average chain length, which may be used for either cotton or wood cellulose.

## EXPERIMENTAL METHODS

**MEASUREMENT OF VISCOSITY.** All the viscosity measurements were made at  $20.00^\circ \pm 0.015^\circ C$ . This degree of accuracy of temperature control is considered necessary for suitable accuracy of the measurements themselves because Triton F has a high viscosity with a rather large temperature coefficient.

The viscometer used (2) was constructed according to the detailed specifications contained in British Standard Method No. 188, standard U-tube viscometer No. 2. It was held firmly in a stout rectangular brass frame, which fitted into a frame-holder mounted permanently in the thermostat, and carefully adjusted to the vertical. The meniscus formed by the solution in the viscometer was observed against an illuminated white background.

To permit control of the liquid level in the arms of the viscometer, a U-shaped attachment to the open ends was used. This was arranged so that the solution could be pushed up into one limb by a slight pressure of nitrogen, which pressure could be instantaneously released by turning a stopcock connecting the two limbs. In addition to the convenience of manipulation, this arrangement allowed the solution to be kept under nitrogen during the course of the measurements.

To fill the viscometer, the solution was poured into a 25-ml. Erlenmeyer suction flask, nitrogen being led in through the side arm. The gas pressure was then used to drive the solution through a small-bore glass tube into the right limb of the instrument. The proper volume adjustment was made with a fine pipet after 20 minutes or more in the thermostat.

When a measurement had been completed, the solution (later to be recovered according to a recommended procedure, 12) was poured from the viscometer and the latter washed out with water, followed by chromic acid cleaning solution. When again to be used, the viscometer was washed with water, alcohol, and ether, and dried for 5 minutes by passing a slow stream of filtered nitrogen through it.

The times of flow were measured to the nearest 0.1 second, for a total time of 260 seconds (solvent alone) to about 300 seconds (cellulose solutions). Each measurement was repeated until 3 or 4 consecutive readings were obtained, which did not deviate from one another by more than  $\pm 0.1$  second.



**PREPARATION OF THE SOLUTIONS.** The most convenient method for dissolving cellulose in Triton F, at the very low concentrations required for chain-length measurements, has been found to be that of Mease (10), utilizing a glass-stoppered bottle containing a glass plunger which on rotation serves to break up the gels sufficiently so that solution takes place. Thus the solution may be kept under nitrogen at all times. Furthermore, in the absence of any stirring device, accurate weighing of the solution is facilitated.

In these experiments, the dissolving vials used had a capacity of about 11 grams of Triton F cellulose solution, which is a little more than is necessary to fill the viscometer. The ground stoppers were of interchangeable standard taper  $\frac{1}{16}$  19/17. For rolling, each vial was fixed into a wide-mouthed container of suitable size. This container and contents were placed on rollers adjusted to make about 1 turn in every 10 seconds. Overnight or about 17 hours' rolling at 25° to 30° C. was necessary safely to complete solution of wood cellulose at the concentrations used.

**CONCENTRATION OF THE SOLUTIONS.** To obtain an accurate measure of the concentration of cellulose in solution, the following procedure has been convenient: The mixing vial, complete with plunger, was weighed (about 40 grams). About 5 mg. of cellulose were added, and weighed accurately in the vial to the nearest 0.05 mg. After being washed out well with nitrogen, the vial was filled with Triton F, so that only a small bubble of nitrogen remained on top after the stopper was inserted. After rolling, the vial and contents were weighed, and the volume concentration (grams per 100 ml. of solution) was calculated from the previously determined density of the solvent.

The problem of obtaining a representative sample when using such a very small quantity of fiber is of importance. There are in existence methods (8) for thoroughly mixing fibrous materials such as wood cellulose. In the experiments recorded here the samples were obtained from regular pulp sheets by thoroughly dispersing portions of several grams in water, filtering on a sintered-glass funnel, and drying from organic solvents at room temperature. The resulting fluffy mats were sampled at random after thorough conditioning (about 5% moisture).

**MATERIALS.** The wood celluloses used in these experiments comprised both commercial bleached sulfite wood pulps and experimental samples. The standard celluloses were prepared according to the ACS method. The very highest viscosity samples of pure cellulose used were obtained by treating unbleached long-fibered cotton with dilute chlorous acid (pH 4) at 65° C. for 2 hours, washing, extracting with alcohol-benzene, alcohol, and ether, drying in vacuo at room temperature, and finally conditioning in air. Hemlock holocellulose was prepared from thin wood sections (40  $\mu$ ) by successively chlorinating in an evacuated container and extracting with hot 4% monoethanolamine in 95% ethanol; this sample was also dried from solvents at room temperature, using the sequence alcohol-ether-benzene.

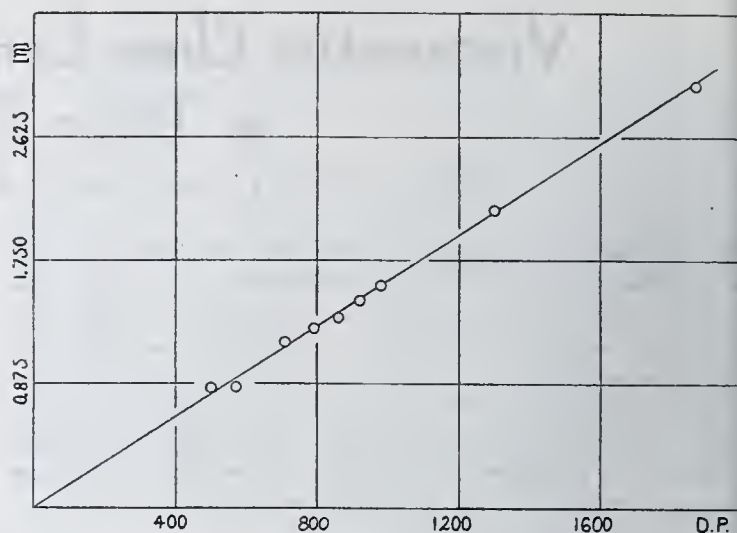
Triton F solution was obtained through the courtesy of Rohm and Haas, and was used as received except for a preliminary filtration (sintered-glass filter) and partial evaporation in vacuo to an apparent strength of 2.10 N, and density 1.086 (20° C.). The characteristics of the solution did not appear to change more than slightly over a period of two years.

#### VISCOSITY AND CHAIN LENGTH

In order to compare different cellulose samples with one another from the point of view of their average chain lengths, by using viscosity measurements in dilute solution, it is necessary to know the form of the mathematical function relating the chain length to the viscosity. In practice, it is also necessary to know the effect of concentration of the cellulose in solution on the measured viscosity. Both functions will depend upon the nature of the solvent used (5).

**Table I. Effect of Cellulose Concentration on Intrinsic Viscosity in Triton F**

Material	10 <sup>4</sup> C, Grams per 100 ml.	10 <sup>3</sup> $\left(\frac{\log \eta_r}{C}\right)$
Standard cellulose from linters	414	1584
	538	1555
	664	1543
	939	1561
	1024	1549
Acetylation linters	271	2015
	494	2060
	1010	2077
Linters for viscose rayon	347	1463
	560	1466



**Figure 1. Effect of Average Degree of Polymerization of Cotton and Wood Celluloses on the Intrinsic Viscosity in Triton F**

If different wood celluloses are to be compared directly, without fractionation into smaller parts of more uniform chain-length, it must be accepted that the chain-length values being used are averages of a special kind, and not necessarily the same average for different solvents. Nevertheless, this fact does not detract from the usefulness of the average values found in a given solvent such as Triton F.

Huggins (5) has recently pointed out the general applicability of the following equation relating the viscosity of high-polymer solutions to the chain length.

$$[\eta] = K (\bar{M})^\nu$$

Here  $[\eta]$  is the intrinsic viscosity, defined by the relation

$$[\eta] = \left( \frac{\ln \eta_r}{C} \right)_{C \rightarrow 0}$$

the relative viscosity,  $\eta_r$ , being the ratio of the viscosity of the solution of concentration  $C$  (grams per 100 ml. of solution) to that of the pure solvent. For nonhomogeneous samples,  $\bar{M}$  is the "viscosity-average" molecular weight, which normally is much higher than the "number-average" molecular weight obtained by osmotic pressure methods. The power  $\nu$  has some value between zero and 2, depending on the solute-solvent system.

In the simplest case,  $\nu$  is unity, and the equation may be written in the familiar form of Staudinger's rule used by Kraemer and his colleagues (6):

$$\text{D.P.} = k[\eta]$$

It will be shown that this equation fits the available data for celluloses from wood and from cotton, within the experimental error.

In making numerous determinations of solution viscosities of different samples it is often customary to choose some small concentration range in which to work, and then use one of the available methods to obtain  $[\eta]$  (at zero concentration) from the actually measured values  $[\eta]_c$  (measured at a finite concentration,  $C$ ). In the case of most wood celluloses, however, it appears that in the concentration range 0.3 to 0.5 mg. per ml., which it is convenient to use, the value of  $[\eta]_c$  remains substantially constant (Table I), and within the experimental error may be used in place of  $[\eta]$ .

In order to compare the Triton F viscosities with actual mean chain lengths of the celluloses used, the required degrees of polymerization have been obtained by the widely used nitration method (1), in which the trinitrate of cellulose is formed under completely anhydrous conditions and the resulting nitrate viscosity measured in an organic solvent; this has been the method used for determining  $k$  (7) for the system cellulose-Triton F.

Certain limitations of this comparison must be kept in mind. The solution of cellulose in Triton F takes place in an aqueous alkaline medium, whereas the nitration takes place in an anhy-



drous acid. Hence it is obvious from what has already been said regarding the effect of Triton F on points of "latent" degradation in cellulose, that processed celluloses that have been subjected to varying degrees of oxidative purification treatments may be expected to show some differences between acid and alkaline viscometric chain lengths. No doubt this accounts for at least a part of the variability of the experimental points on the  $[\eta]$ -D.P. curve (Figure 1). However, the fact that processed long-fibered cottons and purified linters fit so well into the series of wood celluloses would seem to indicate that the divergency from this cause is not at all serious here. Cellulose nitrate degree of polymerization values are used in the first place because they are possibly the most widely used and fully investigated values at present available.

These results lead to the following relationship:

$$\text{D.P.} = 618 \left( \frac{\log_{10} \eta_r}{C} \right)$$

$C \geq 0.03$  gram per 100 cc. of solution

Triton F 2.10  $N$

Temperature 20.0° C.

#### DISCUSSION OF RESULTS

The advantages of Triton F solution as a cellulose solvent, already discussed, are further emphasized by experience with the method in this laboratory. Without the extreme precautions properly required in using cuprammonium solutions (1), and without the otherwise inconvenient necessity of carrying out a special nitration, a large number and variety of cellulosic samples—largely wood celluloses of an experimental nature—have been examined and compared as to average chain length, with results that have always been consistent and reproducible.

Quaternary ammonium bases are supposed by Licser (9) to disperse cellulose to single molecules in solution. The present viscosity results now provide further indirect evidence to support this view, as the constant relationship between the trinitrate and quaternary base viscosities over a range of chain lengths (Figure 1) seems inconsistent with the view that micellar particles exist to any great extent in the alkaline solutions. Viewed in the darkfield microscope, the solutions of 0.1% concentration appeared completely free of any larger undissolved particles such as gels.

An important source of difficulty found by previous users of Triton F (13) has been the "aging" effect, whereby the solutions of cellulose gradually drop in viscosity with time. The source of this effect has never been definitely ascertained. However, in the case of the very dilute solutions used in the present experiments, the aging effect does not appear to be appreciable. This has been shown by the constancy of results that has been obtained when using different rolling times, no drift being evident even up to 48 hours. Furthermore, the solution kept in the viscometer for a day or more showed no change in viscosity. Thus aging seems to be a characteristic of Triton F cellulose solutions that are moderately or highly concentrated.

The effect of the concentration of quaternary ammonium base on the specific viscosity of the cellulose dissolved in it has not been examined. According to Russell and Hood (12) the specific viscosity falls with increasing normality of base. The viscosity of the aqueous base itself changes very rapidly with concentration in solutions strong enough to be used as cellulose solvents.

The technique of viscosity measurement described is not such as readily lends itself to large-scale commercial control practice in a pulp mill, as preparing and weighing the small sample used requires a rather high degree of precision. As a research tool in the field of cellulose chemistry it would appear to have worthwhile advantages. However, the method could possibly be used to advantage as a supplement to routine control practice.

Thus, in technical control work the customary concentrations of wood pulp used run from 1 to 5% in the cuprammonium re-

agent. However, it is well known that these viscosities have no real meaning in terms of average chain length, particularly in the case of high-viscosity pulps, since what is being measured is a "structural viscosity" (11) dependent to a high degree upon many extraneous factors. (The data of Table II offer an illustration of this.) In fact, the cuprammonium viscosity as ordinarily obtained can only be regarded as a cellulose quality factor, reflecting average chain length to some extent but reflecting also to an inordinate degree such factors as the chain-length distribution in the  $\alpha$ -cellulose fraction, the nature of any retained hemicelluloses, and even the presence of inorganic contaminants. In dilute solution the effect of any of these factors is relatively small or nonexistent, the controlling variable being the weight or viscometric-average chain length.

It seems apparent therefore that the determination of Triton F viscosities in dilute solution, leading to a value of the average chain length, offers definite advantages for mill control practice as an occasional check on the routine cuprammonium viscosity values, especially when as sometimes happens, these seem to vary in an unexpected manner. The knowledge that the degree of polymerization of a pulp is, or is not, abnormal should prove most helpful in such cases, in view of the many other variables affecting the cuprammonium value.

Table II. Comparison of Triton F and Nitrate Viscosities of Wood and Cotton Celluloses

Cellulose Sample	Cuprammonium Viscosity, Cp.	Triton F $10^3 \left( \frac{\log \eta_r}{C} \right)$	Nitrate D.P. <sup>a</sup>
No. 1 wood cellulose	1.43	850	500
No. 2 wood cellulose	1.93	850	570
Std. cellulose from linters	2.10	1170	710
No. 3 wood cellulose	4.08	1278	790
No. 4 wood cellulose	15.2	1340	860
Linters A	3.72	1460	920
Linters B	4.59	1582	980
Linters C	11.4	2119	1300
Purified cellulose from cotton	...	3000	1875

<sup>a</sup> Determined in 0.05% ethyl acetate solution (D.P. = 270  $[\eta]$ ).

Table III. D.P. Values Obtained in Triton F Solution for Typical Cellulose Samples

Rayon-grade bleached sulfite pulps	500-800
99% $\alpha$ -cellulose bleached sulfite pulp	1135
High-viscosity sulfite pulp	1460
Long-fibered cotton, bleached	1580
Holocellulose, from western hemlock, after weak alkaline extraction at room temperature	1640

#### ACKNOWLEDGMENT

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# Water Vapor Permeability of Organic Films

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An apparatus for a rapid, precise, and reproducible determination of water vapor permeability is described. It seems that two factors, diffusion velocity and solubility, together determine the vapor permeability of a film. Both can be derived from the measurements. Equations based on the validity of Fick's law are tested with commercial films of low permeability. The dependence of permeability on thickness, vapor pressure, and temperature is studied. On the basis of measurements of a number of films a discussion of the process of permeation is presented.

**A** MOST important determining factor in the selection of an apparatus with which to measure water vapor permeability is the amount of water that will pass through a film of convenient size and thickness in a relatively short time. For recently developed films that have very low water vapor permeability as compared with previous commercial samples, about  $10^{-6}$  to  $10^{-7}$  gram of water will pass through a film with an area of about 25 sq. cm. in about an hour under a pressure gradient of about 20 mm. A good MacLeod gage can be adapted to measure quantities of this magnitude with a precision between 5 and 10%. An apparatus based on these considerations is described below. In addition to furnishing a rapid and reproducible absolute measure of the permeability of films, it is also possible to obtain from the same measurement the values of two constants which together determine the amount of water transported through the film under given conditions.

In most cases, as long as mechanical injuries do not cause breaks in the film, the transmission of water vapor can be represented as a process of activated diffusion which has been treated by Daynes (5) and Barrer (1, 2).

## THEORY OF PERMEABILITY

**UNITS.** The amount of water vapor,  $Q$ , which will pass through a given film at a given temperature depends upon the area,  $a$ , the thickness,  $l$ , the vapor pressure difference,  $\Delta p$ , and the time,  $t$ , according to the following steady state equation (1, 2):

$$Q = P \frac{a}{l} t \Delta p \quad (1)$$

$P$ , the proportionality factor, is termed the permeability constant and characterizes the resistance to water vapor transmission for the material under consideration. The dependence of  $Q$  upon the area and the time as expressed in Equation 1 is unquestionable. The validity of the linear dependence of pressure and the inverse linear dependence of thickness are discussed below.

For our purposes, we define the permeability constant as the cubic centimeters of gas at standard temperature and pressure passing per second through a membrane 1 sq. cm. in area and 1 mm. thick when the pressure difference is 1 cm. of mercury. Thus Equation 1 becomes the defining equation, giving  $P$  the following units<sup>1</sup>:

$$P = V \frac{l}{\Delta p a t} = (\text{cc. of gas}) \frac{\text{mm.}}{(\text{cm. Hg}) \text{ sq. cm. sec.}} \quad (2)$$

Thus the value of  $P$  will rate the film material relative to others for water vapor transmission and will also provide data necessary to calculate the amount of water that will pass through the film in a given time if the other constants in Equation 2 are known.

Fick's law (compare 1) states that the rate of transmission per unit area of a gas through a solid in the direction of the concentration gradient of the gas  $\partial c / \partial x$  is given by

$$P' = D \frac{\partial c}{\partial x} \quad (3)$$

where  $D$  is the diffusion constant.  
Using Henry's law

$$c = S p \quad (4)$$

to express the concentration in terms of the pressure Equation 3 becomes for a film of thickness,  $l$ , in a state of steady flow

$$P' = D S \frac{P_2 - P_1}{l} = D S \frac{\Delta p}{l} \quad (5)$$

The diffusion constant,  $D$ , is the cubic centimeters of gas at S.T.P. passing per second through a centimeter cube of the material when there is a unit concentration gradient across the cube. We define  $S$ , the solubility coefficient in Henry's law, as the cubic centimeters of gas that will dissolve ideally in 1 cc. of film material when the pressure of the gas is 1 cm. of mercury. Thus  $S$  has dimensions of cc. of gas per cc. of film material per cm. of mercury. Since  $P'$ , the permeation rate constant, has units of cc. of gas per second per sq. cm., the permeability constant,  $P$ , differs from the permeation rate constant,  $P'$ , by a factor  $l / \Delta p$ .

In Equation 2  $l$  was measured in millimeters; in Equation 5 in centimeters; consequently, a factor 10 must be included to correct for this. That is,

$$P = 10 P' \frac{l}{\Delta p} \quad (6)$$

Substituting Equation 5 for  $P'$  gives

$$P = 10 D S \quad (7)$$

which relates the constants defined above.

Consequently, the permeability of a film can be broken down into two contributing factors, the diffusion constant and the solubility coefficient. The first quantity measures the probability that each individual gas molecule will move in the direction of the concentration gradient, the latter is a measure of the number of the gas molecules per unit volume which contribute to this

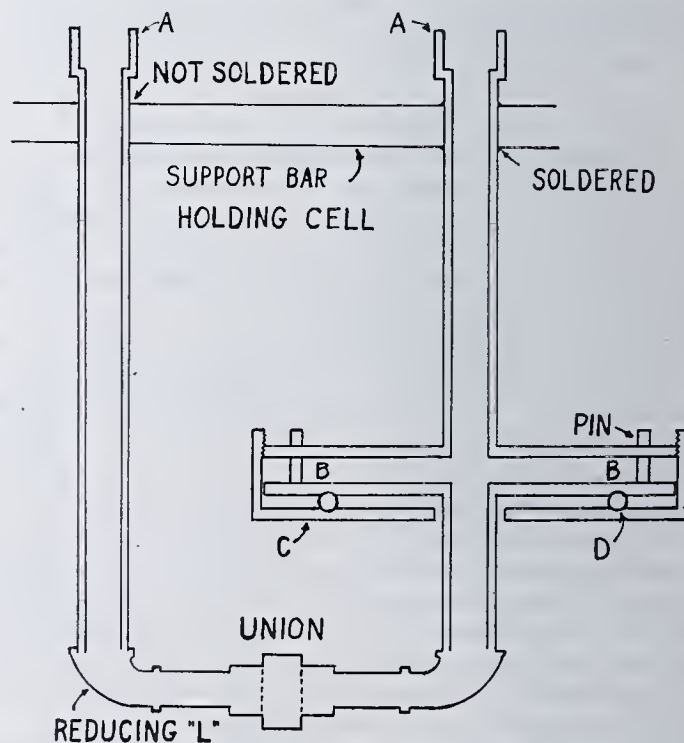


Figure 1. Permeability Cell

<sup>1</sup> It seems to the authors that Barrer has misplaced the mm. unit in this definition (1, 2).



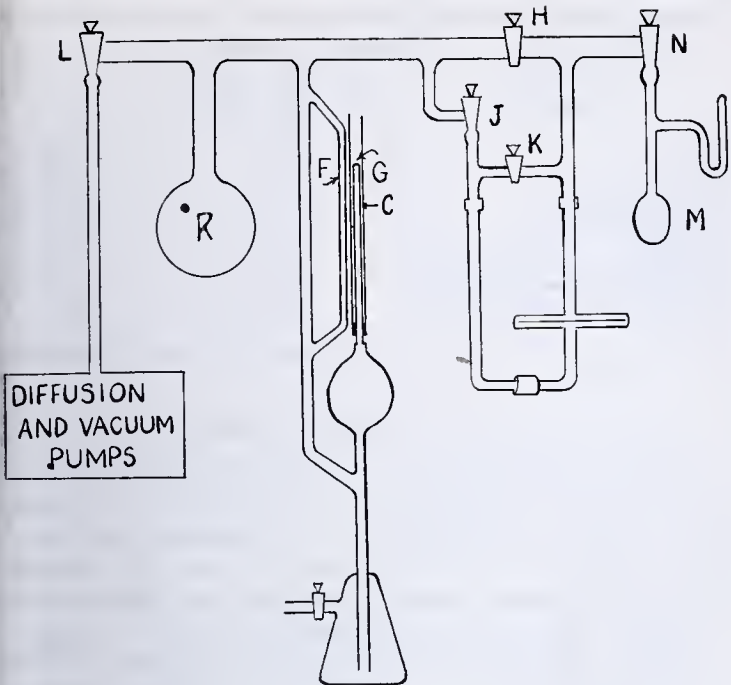


Figure 2. Vacuum System

concentration gradient. The solubility coefficient does not include the water molecules that are sorbed but only those that are ideally dissolved and consequently this may or may not approximate the actual equilibrium solubility.

APPARATUS AND MEASUREMENTS

**DIFFUSION CELL.** A detailed diagram of the diffusion cell (13) is shown in Figure 1. The cell is made from brass with all permanent joints silver-soldered. The enlarged ends, A, provide for sealing in glass tubing with Picein or deKhotinsky cement. A more permanent metal-glass joint is not used because the seal is occasionally broken while opening or sealing the cell. With a Picein joint a short heating will reseal the joint, whereas a copper-glass joint must usually be entirely replaced. (A similar cell made from Pyrex glass has recently been put into operation successfully.)

In the cell, B, rubber jar rings are used for gaskets without lubrication. They may be sealed to the brass plates with rubber cement above and below B in Figure 1. Copper gauze is laid under the film for even support.

The cap, C, is screwed upward to seal the membrane tightly in the cell. Ball bearings, D, are used between the cap and the cell. It is best to have these in a groove with a spacer. The union is fitted with a washer cut from gasket rubber—i.e., rubber with wire screening embedded in it. Both the union and cell need only be tightened with moderate force to secure high-vacuum-tight seals.

**VACUUM SYSTEM.** The cell is mounted in a vacuum system as shown in Figure 2. It is essential to have a high-quality MacLeod gage. Water vapor pressure can reliably be measured with the MacLeod gage in the following manner: Surround the capillary with a water jacket as shown in Figure 2. To make a reading allow the mercury to rise above the cutoff point. With a cool flame heat the bulb and the water jacket, then allow the mercury to rise slowly until the mercury in capillary F is opposite the top of capillary G. The pressure at 0° C. (which is used in the calculations) is given by

$$P = Ch^2 \frac{273}{273 + T}$$

where C is the MacLeod gage constant, h is the distance in millimeters from the top of capillary G to the mercury column, and T is the temperature of the water in the water jacket, C. T must be high enough so that the vapor pressure at temperature T is greater than h.

All stopcocks are high-vacuum type using Apiezon "L" as lubricant. When stopcocks H and J are closed, a vacuum of 10<sup>-6</sup> mm. should be obtained. When they are opened to the newly mounted cell the pressure will read about 5 × 10<sup>-4</sup> mm. if there are no leaks. Pumping for several hours with the thermostat at

about 70° is necessary to reduce the outgassing of the metal to such a degree that with pumping a vacuum of 10<sup>-6</sup> mm. is obtained.

The volume of the system into which the water vapor diffuses must be measured. This volume, which should be about 4 liters, is conveniently obtained by means of an auxiliary bulb of known volume connected to the system by means of a stopcock.

The water in flask M is frozen and evacuated several times to remove air. The apparatus is now ready for use.

**METHOD OF MEASUREMENT.** Stopcocks H and J are closed; the cell is opened by unscrewing the cap, C, and the union. The film is put in place and the cell and union are screwed shut tightly. Now stopcock K is opened to prevent unequal pressure on the film when H and J are opened. With the pumps going and L open, H and J are opened. Pumping is continued until pressure is 0.01 to 0.03 micron (about half an hour). After this time, a blank is usually run—that is, stopcocks H, K, and L are closed and the pressure is measured at 5-minute intervals for four or five measurements. This pressure-time plot should give a straight line. Then H is opened for a short time while the temperature of the water in M is adjusted to give the vapor pressure of water that is desired. To begin the measurement L and H are closed and N is opened. The pressure is then measured as described above every few minutes. Measurements are continued until the data give a straight pressure-time plot.

**METHOD OF CALCULATION.** The pressure increase during the run must be corrected by subtracting from it the pressure increase measured as the blank. When the corrected pressure is plotted versus the time a graph similar to Figure 3 is obtained. The permeability constant, as defined above, is obtained by using measurements from the graph in the formula:

$$P = \left[ p(\text{mm.}) \frac{V}{760} \right] \frac{l}{a \Delta p T} \tag{8}$$

where p(mm.) is the pressure in millimeters of some arbitrarily chosen point near the end of the plot. V is the volume in cubic centimeters into which the gas expands after diffusing. l is the thickness in millimeters and a is the area in square centimeters. Δp is the pressure of water vapor and T is the portion of the time axis as shown.

The diffusion constant, D, is obtained from

$$D = \frac{l^2}{6L} \tag{9}$$

which is according to Barrer (1) the solution of the general Fick's law equation under the existing boundary conditions. l is in centimeters and L is the time intercept in seconds.

The solubility coefficient is obtained from Equation 7.

**EFFECT OF HOLES.** If there are capillary holes in the film which contribute to its over-all permeability, some water will be transmitted immediately. Thus, the pressure versus time plot will not have an induction period where there is no rise of pressure, but rather the plot will leave the origin in a straight line at an angle

Table I. Water Vapor Permeability of Film Materials<sup>a</sup>

Film Material	P × 10 <sup>3</sup>	D × 10 <sup>8</sup>	S	Thickness, Mm.
Cellophane (TAPPI standard sample)	4.7	0.39	1.2	0.035
Cellophane, specially coated	5.4	0.051	10.6	0.031
0.1-mil coating	0.25	0.36	0.070	0.036
0.4-mil coating				0.021
Koroseal (see Table II)	5.6	0.39	1.8	0.175
Av. value	3.7	0.20	1.8	0.045
Nylon I	260	1.1	24	0.034
II	6.0	0.28	2.1	0.022
III	14.2	0.25	5.5	0.051
IV				
Pliofilm (TAPPI standard sample)	1.3	0.15	0.88	0.082
Polythene	2.1	0.48	0.44	0.041
Saran (a)	0.070	0.125	0.06	0.026
Saran (b) (three measurements)	0.135	0.11	0.13	0.026
Vinylite I, calendared	470	2.3	20.5	0.102
Vinylite II, cast	37	20	0.19	0.107
Waxed glassine	0.45	0.54	0.083	0.045
3-Ply glassine	1.2	1.2	0.10	0.083
Ethylcellulose	510	19.3	2.6	0.505

<sup>a</sup> Measurements made at 25° C. and 20-mm. vapor pressure (77° F. and 85% relative humidity).



to the time coordinate. In other words, there will be superimposed upon the ordinary graph (Figure 3) a straight line through the origin. The amount of permeation due to pin holes can be estimated from this.

### RESULTS

A number of results that have been obtained are presented in Table I. These results apply only to the actual films measured. There is such a variation in the permeability of commercial films of the same trade name that it would be incorrect to attach to any class of films the specific value recorded below, which is based on the measurement of only a few film samples. All measurements were made at 25° C. Roman numerals designate film materials of different composition but same commercial name. Letters refer to different measurements on a different portion of the same film sample.

Table II. Effect of Thickness on Permeability Constant

Thickness, Microns	$P \times 10^8$
Koro seal	
21	7.4
34.5	6.9
47	4.6
99	6.4
134	4.5
150	4.1
Polythene (Cast)	
58	2.38
105	2.46
163	2.40

### DEPENDENCE OF PERMEABILITY ON THICKNESS

Equations 1 and 2 state that the permeability is inversely proportional to thickness and that the permeability constant is independent of thickness. This is not true for films containing hydrophilic material—e.g., natural rubber (16)—or for films made from materials that sorb much water—e.g., cellulose.

On the other hand, numerous cases have been recorded where Equations 1 and 2 are valid (3, 4, 6, 9, 14, 15, 16).

The authors have extensively studied a series of samples of different thicknesses made in an identical manner from the same material, furnished through the courtesy of the B. F. Goodrich Company. Table II shows the results of repeated determinations of the permeability constants of this film in different thicknesses.

These values of  $P$  could be checked within 0.5 unit; consequently, the fluctuations of  $P$  were not due to fluctuations in measurements but rather to actual differences in the films. However, over a sevenfold range in thickness the permeability constant showed no significant trend. The necessity of using many samples of different thicknesses in this type of study should be appreciated, for different conclusions could have been drawn if only two of the above samples had been measured. A similar study on films prepared from cast polythene showed no change of permeability constant over the thickness range studied. The results are summarized in Table II.

### EFFECT OF PRESSURE

The quantity of water passing through a film may or may not be directly proportional to the vapor pressure difference. The direct proportionality does not exist in films which contain hydrophilic material or polar groups that sorb water molecules (16). However, Equations 1 and 2 may be considered as the ideal law of gas permeability, which, for many films (particularly for those of low permeability) adequately represent the effect of thickness and pressure difference. Deviations from this ideal behavior usually begin to become significant when the film material sorbs water noticeably. This is particularly apparent in the case of cellulose sheeting. In Table III are presented some measurements of permeability constants at different pressure differences. All measurements are at 25° C.

Table III. Effect of Vapor Pressure on Permeability Constant

Material	Pressure Difference, Mm. Hg	$P \times 10^8$
Saran	15	0.16
Saran	20	0.14
Saran	24	0.15
Pliofilm, laminated	10	0.11
Pliofilm, laminated	18	0.11
Vinylite III	4.6	37.8
Vinylite III	20	38.0
Cellulose (uncoated cellophane)	4.6	3.3
Cellulose (uncoated cellophane)	6.5	4.7
Cellulose (uncoated cellophane)	10	68
Cellulose (uncoated cellophane)	20	84

### EFFECT OF TEMPERATURE

Barrer's measurement of permeability constants for permanent gases at different temperatures (1, 2) is direct proof of transmission by activated diffusion, for the temperature dependence is clearly exponential. However, the authors have been unable to find in the literature reliable measurements of the temperature variation of water vapor permeability. In Table IV the permeability constants for single-ply pliofilm (PID 140) at different temperatures are shown. Figure 3 shows the great difference in permeability at different temperatures. The log  $P$  versus  $1/T$  plot (compare Figure 4) gives a reasonably straight line, showing that the data can be represented by

$$P = P_0 e^{-E/RT} \quad (10)$$

The energy of activation in this case is about 13,000 calories per mole, diffusing water vapor.

From these measurements and those of Barrer, one may say that in general the permeability of a good film approximately doubles for a 10° C. (18° F.) rise in temperature.

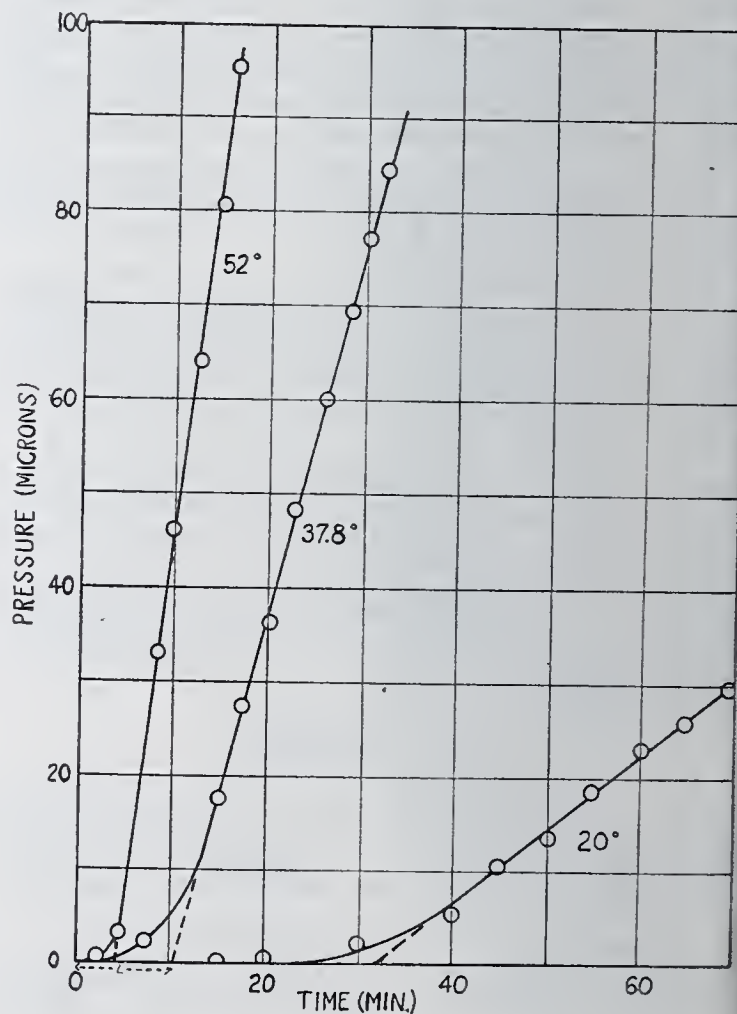


Figure 3



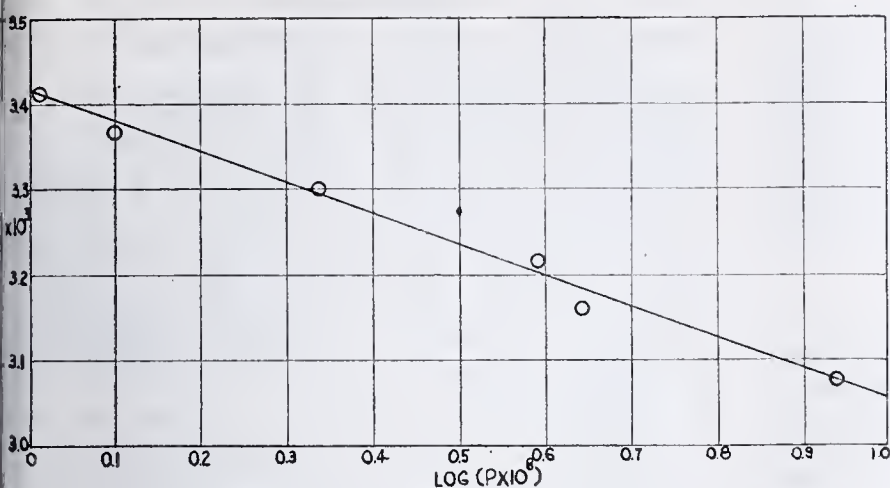


Figure 4

Table IV. Effect of Temperature on Permeability Constant

T, ° C.		P × 10 <sup>3</sup>
20	Pliofilm	1.03
24		1.26
30		2.1
37.8		3.9
43.5		4.4
52		8.7
30	Vinylite IV	58
38		74
25	Polythene	2.38
30		3.57

## PROCESS OF PERMEATION

On the basis of the foregoing observations and measurements, seems that the transport of small molecules, such as water, nitrogen, oxygen, hydrogen, etc., through a layer of a polymer can be visualized by two different mechanisms: (1) flow through capillary holes, and (2) activated diffusion through the polymer itself.

The first process involves the presence of a system of preformed revices or capillaries, the average width of which is larger than the diameter of the diffusing molecules.

Under the influence of its random kinetic motion, an individual molecule eventually will enter into such a capillary. It is then irregularly reflected at the capillary walls and carries out a Brownian movement very similar to the one characteristic for the gaseous state. Whether the net effect of this "free" diffusion will be better described by a Poiseuille flow or by a Knudsen diffusion depends upon the ratio between the mean free path of the molecules and the average diameter of the capillaries. This in turn will be influenced by the vapor pressure of the diffusing gas and the porosity of the polymer. After having been frequently reflected at the walls of the capillaries, the migrating particle finally emerges on the other side of the layer and one elementary step of gas transport through the polymer has taken place. This unactivated diffusion of small molecules through existing crevices along surfaces of crystallites, between morphological elements, such as fibrillae, etc.) is only moderately temperature-dependent; it is proportional to  $T^*$  where  $a$  assumes values around 0.5, depending upon the shape of the diffusing molecules and of the character of the capillaries. The net effect, which this mode of transport contributes to the total penetration of the gas through the film, is proportional to the pressure gradient, the time, and the area, and depends in a somewhat complicated manner upon the size and shape of the diffusing molecules and of the channels through which they travel.

The activated diffusion of molecules through the bulk of the polymer itself is of an altogether different character.

Let us assume first for simplicity that the film through which the water is supposed to migrate is homogeneous and in a completely disordered (amorphous) state. It can then be considered to be a liquid of very high viscosity or a glass. Water molecules of the gas space will, under the influence of their random kinetic

motions, hit the boundary plane of the film; in many cases they will be reflected elastically, in others they will penetrate into the first few atomic layers of the polymer and then start to diffuse irregularly through the material. Their motion will not (as in the first case) be comparable with the conditions in an ideal gas, but rather to the Brownian movement of a particle which is dissolved in a very viscous liquid. Using the concepts of the viscosity of liquids and particularly of long-chain substances as developed recently by Eyring and his collaborators (7, 8), one arrives at the conclusion that the migrating molecule carries out quasi-elastic vibrations around a certain position, until through the segment movement of the surrounding chains a "hole" is formed in its immediate neighborhood and it moves into this hole, where it vibrates again for a period, long as compared with the time of transition from one hole to the next. Thus the dissolved particle moves through a series of holes, though they are not pre-existent but are gradually produced (and disappear again) by thermal vibrations of the segment inside of the polymeric material. The probability

for the formation of such a hole is comparatively small and the molecule has therefore to wait comparatively long in one hole until another one is formed in its immediate neighborhood into which it eventually can move. Thermodynamically, the holes are characterized by a certain energy and entropy of formation and these two quantities can be derived from observation of the rate of this "activated" diffusion and from its temperature dependence. The order of magnitude of these two quantities (expressed for one mole of holes) is for water and pliofilm about 12,000 calories per mole for the energy and 10 calories per degree for the entropy of activation.

Finally, there exists a third process for the transportation of a molecule through a polymer, which, in a certain sense, involves a mechanism intermediate between the two mentioned above.

It has been known for some time (10) and has been confirmed and considerably elaborated recently (12, 14) that many high polymers, such as cellulose and its derivatives, possess a so-called internal surface, which is accessible for gas molecules and can be measured by adsorption studies. This suggests the existence of very narrow slits and crevices, the walls of which contain a large number of centers of high adsorption power (active spots or points of high intermolecular attraction). In the case of the diffusion of water through cellulose, the hydroxyl groups on the surface of the cellulose crystals presumably are such points of high water-binding capacity. If a water molecule enters such a slit between two crystals, it will be immediately adsorbed at one of these hydroxyl groups and after the heat of adsorption has been dissipated, carry out vibrations in the immediate neighborhood of this group, until by thermal fluctuations it acquires the energy necessary for desorption. It will then re-evaporate into the slit and after a few elastic (inefficient from an adsorption standpoint) collisions with the wall, be adsorbed by another active spot, where it stays for another period, and so on. This process can also be considered to be an activated diffusion, but a diffusion through a preformed slit or system of holes. It is characterized by an energy and entropy of activation, but these two quantities have now another significance than before. The energy of activation (between 8000 and 14,000 calories per mole of water) is the energy necessary to evaporate an adsorbed water molecule from the active spots, while the entropy of activation involves the difference in the modes of vibration and rotation which an individual molecule carries out in the free and adsorbed states, respectively. In this case, both quantities have nothing to do with the irregular segment motion (micro-Brownian movement) of the polymer, but with the specific attraction between the diffusing molecules and certain active groups, which are distributed on the walls of the slit in the polymers.

The real effect of crystallinity on the permeability of high polymers has not yet been investigated and there exist widely different opinions about the relative importance of the crystalline and amorphous areas in a polymer. In this consideration the work of Langmuir and Schaefer (11) seems to be of great importance. They found that a single monomolecular crystallized layer was sufficient to cut down the rate of evaporation of water by a factor of about one million.

On the basis of permeability measurements alone it does not seem easy to decide whether the actual experimental qualities



(and their formal interpretation as described above) point to an activated diffusion through gradually forming and disappearing holes or along the surfaces which are covered with active spots. However, it appears that additional information about the polymer, such as the presence of active groups (hydroxyl, amino, carboxyl, carbonyl, etc.) or the crystalline character of the material and the existence of an internal surface, together with more numerous and more precise measurements, may help in the elucidation of the true permeation process.

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## Evaporation Indices of Hydrocarbon Thinners

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ONE of the most distinctive and characteristic properties of a solvent naphtha is its rate of evaporation. Upon this evaporation rate of the solvent itself depends its choice for application to the great variety of organic materials which must be thinned prior to use. The factor of "solvent retention" does modify the evaporation rate of the solvent itself, but contributions of the solute are generally constant in so far as they affect the rates of solvent release of interchangeable solvent naphthas.

It is rather astonishing, therefore, to reflect that until very recently no concerted effort has been made to standardize an acceptable procedure to measure evaporation rate. A number of evaporimeters have been described in recent years, but in nearly every case their use has been confined to their parent laboratories (2, 3, 9-14). This state of affairs is aptly epitomized in a report of the (British) Institute of Petroleum (8):

An attempt has been made to standardize a suitable relative evaporation test for the examination of solvents to be used in the paint and lacquer industry. From contact with paint research stations and industrial firms, it appears that no suitable well-recognized test is in existence. Those that are actually used are either simple rule-of-thumb tests or require complicated apparatus and are not suitable for routine work. As it is generally agreed that such a test is of value in assessing the behaviour of solvents, advice on the methods used in the U.S.A. was sought from the A.S.T.M., but so far the appropriate committee of that body is taking no steps to standardize a test, as they have not been asked to do so by their members. In the meantime a search is being made of the literature available on the subject, so that the significance of any method can be fully appreciated.

The subject of evaporation rate is now under consideration by the A.S.T.M. The problem of devising a generally satisfactory method, however, requiring inexpensive but accurate and reliable equipment, would appear extremely difficult. Neither volumetric nor gravimetric methods lend themselves readily to operation in a water thermostat; and only a handful of supplier and consumer laboratories are air-conditioned at the present time. Few, if any, gravimetric methods provide for unchanging air flow in the vicinity

of the evaporating liquid. Nor is it a simple matter to charge a gravimetric surface with a highly volatile liquid sample without substantial evaporation during the time required for charging. Volumetric apparatus, such as that of Wetlaufer and Gregor (14) or the modification employed in the present work, must be up-ended frequently for periodic readings, thus introducing errors due to variation in surface tension, completeness of drainage, and ratio of vapor to liquid volume. Evaporation of solvent naphthas from their solutions of a "standard" vehicle—e.g., varnish-grade linseed oil—would introduce this limiting factor.

In view of these difficulties, an attempt has been made to estimate the evaporation rates of commercial solvent naphthas, from control tests which are normally run on regular shipments—viz., their specific gravities and their volatilities as measured by A.S.T.M. distillation (1).

#### EVAPORATION INDICES

As generally plotted, evaporation rate curves represent absolute values for one set of conditions. Presumably, however, slight

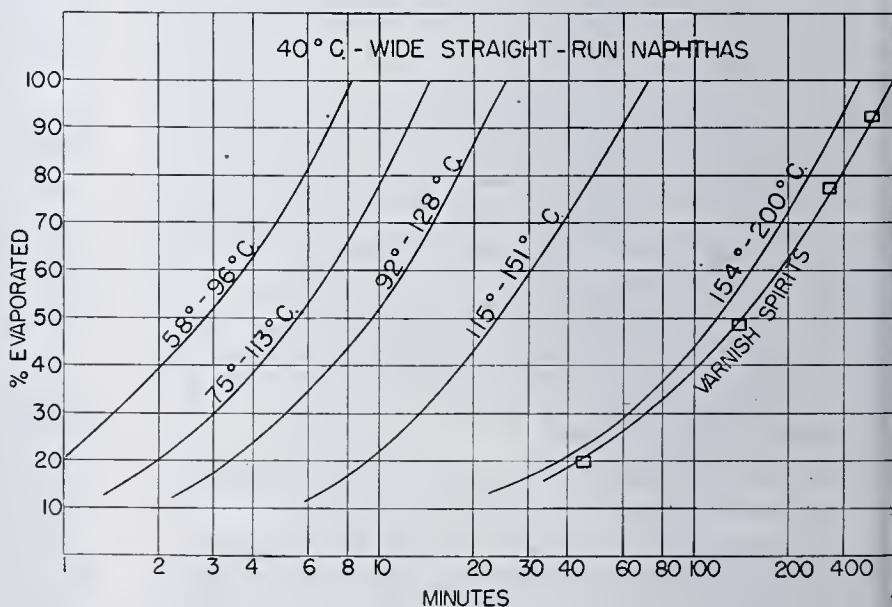


Figure 1



Table I. 40° C.-Wide Straight-Run Petroleum Solvent Naphthas

	Narrow-Cut Rubber Solvent	Extraction Naphtha	Lacquering Diluent	Narrow-Cut V.M. & P.	Mineral Spirits	Varnish Spirits
Boiling range, ° A.P.I.	71.0	64.0	60.0	55.8	49.0	43.9
Specific gravity at 60°/60° F.	0.702	0.724	0.739	0.756	0.784	0.807
Initial distillation temperature, ° C.	58	75	92	115	154	154
% off	73	90	105	129	170	172
End point	96	113	128	151	200	198
Evaporation time at 25° C., minutes						
20%	1.0	2.0	3.4	9.5	39	43
50%	2.8	5.5	9.6	24	120	143
80%	5.8	10	17.7	47	232	300
95%	7.6	13.6	23.5	65	310	400

variations in procedure or surroundings will produce the same relative, or comparative, values within a series of naphthas, if one member is run identically. It thus appears likely that if a complete series can be run once under optimum conditions, and if an actual or hypothetical member be taken for reference, further if a satisfactory graphical or mathematical relation is established between the reference naphtha and the other members of the series, no further "control" evaporation runs need be made. After the curve for the reference naphtha is arbitrarily plotted, the other members may be described by, say, four points—e.g., their 20, 50, 80, and 95% evaporation times. Knowing these particular points, adequate curves may be plotted, and also information gained concerning their contribution to the behavior of freshly deposited coating films. For example, at the point of 50% evaporated, the coating film viscosity may be increased to, or beyond, that necessary for "setup"; at 95%, nonoxidizing films may have become dust-free; while at 20% evaporated such films are frequently touch-dry (4).

Using the data here presented, it is proposed to refer all evaporation times to those of a hypothetical straight-run petroleum naphtha of roughly 40° C. boiling range, and with a 50% A.S.T.M. distillation temperature of 80° C. Then the 20% evaporation time of any actual naphtha, divided by the 20% time of the reference naphtha, will be termed the 20% evaporation index, and is written  $I_{20}$ .

#### NARROW-CUT NAPHTHAS

Except for wide-cut rubber solvents, where a 100° C. boiling range is needed for quick setup and long tack time, the great majority of volatile straight-run petroleum thinners boil within 40° F. (50° C.), and of these nearly all except the "mineral spirits" cuts boil within 40° C. Table I lists a representative series of widely used straight-run naphthas, with 50% distillation temperatures from 73° to 172° C.—i.e., with volatilities covering ordinary requirements. Except for the two mineral spirits which have a 45° C. boiling range, the members of the series boil within 40° C.

With the exception of the "varnish spirits", all items in Table I are distilled from paraffinic crudes, and as a result have specific gravities corresponding to a large paraffinic content. Paraffinic naphthas contain minor proportions of aromatics and naphthenes, but are free from olefins; and it can be generally stated that the average domestic paraffinic crude will produce straight-run naphthas whose paraffinic content gradually decreases with increasing boiling range. For a given volatility, the specific gravities shown in Table I are close to those of the majority of 40° C.-wide paraffinic naphthas generally available. Specific gravity will therefore be taken into account when we are dealing with straight-run naphthas of the paraffinic type.

#### EVAPORATION RATE CURVES

The evaporation data shown in Table I are plotted in Figure 1 on semilog paper. For the time being, the nonparaffinic varnish spirits will be neglected.

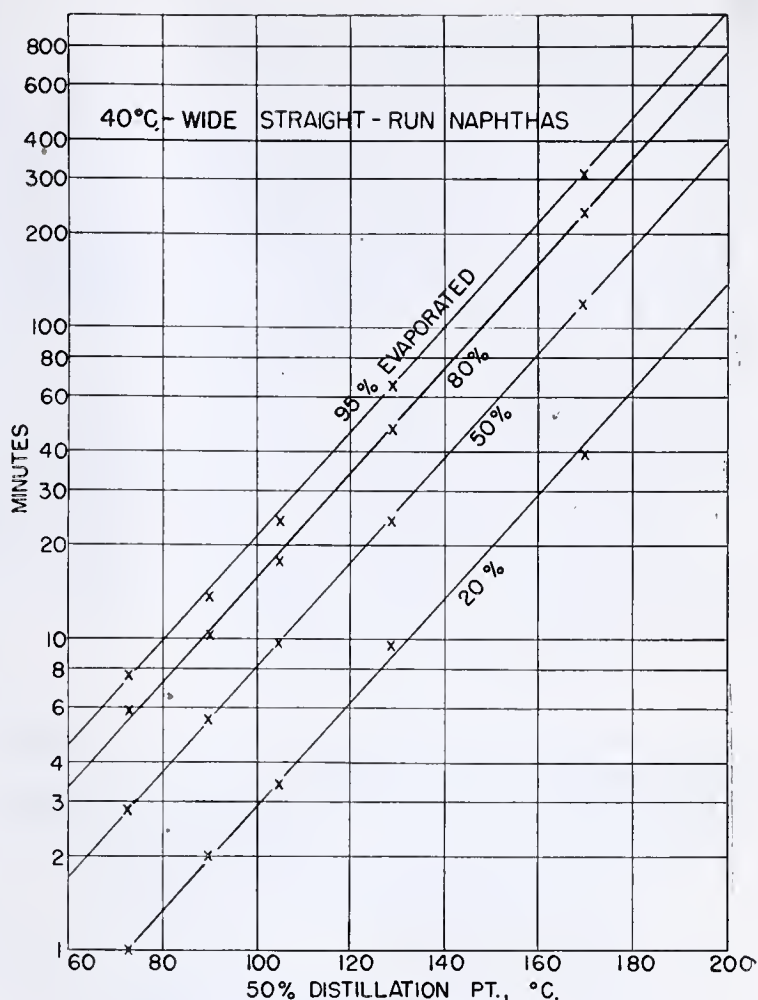


Figure 2

If we now plot, again on semilog paper, the 20, 50, 80, and 95% evaporation points of each of the five narrow-cut straight-run paraffinic type naphthas against their 50% A.S.T.M. distillation temperatures, we have the family of parallel curves shown in Figure 2. Here evaporation time,  $t$ , in terms of 50% distillation temperature,  $T$ , in ° C., is given as follows:

$$\begin{aligned} \log t_{20} &= 0.01675T - 1.22 & \log t_{80} &= 0.01675T - 0.48 \\ \log t_{50} &= 0.01675T - 0.77 & \log t_{95} &= 0.01675T - 0.36 \end{aligned}$$

In the case of the hypothetical 40° C.-wide reference naphtha, with 50% distilled at 80° C., we have the following evaporation times: 20% in 1.32 minutes; 50% in 3.7; 80% in 7.2; and 95% in 9.5.

Evaporation indices of actual 40° C.-wide paraffinic type naphthas,  $I_{20}$ , etc., now become  $\frac{t_{20}}{1.32}$ , etc., or, from the above equations:

$$\begin{aligned} I_{20} &= 0.76 \text{ antilog } (0.01675T - 1.22) \\ I_{50} &= 0.27 \text{ antilog } (0.01675T - 0.77) \\ I_{80} &= 0.138 \text{ antilog } (0.01675T - 0.48) \\ I_{95} &= 0.105 \text{ antilog } (0.01675T - 0.36) \end{aligned}$$

#### WIDE-CUT NAPHTHAS

Data on wide-cut naphthas are shown in Table II. These include a rubber solvent, an extraction naphtha, and a varnish makers' and painters' naphtha. The first two naphthas, with boiling ranges of 100° C., evaporate much faster than 40° C.-wide naphthas of the same paraffinic 50% A.S.T.M. distillation temperatures. They nevertheless exhibit closely similar curvatures in their evaporation rate curves, and, as a result, a simple correction can be made by subtracting 13° C. (23° F.) from the 50% distillation temperature of the 100° C.-wide naphtha. Figure 3 shows the excellent concordance of the test data with this assumption.

Another exception to the 40° C.-wide line of naphthas is wide-cut V.M. & P., usually boiling from 210° to 320° F.—i.e., 60° C. wide. Here, evaporation rate is estimated by subtracting 8° C.



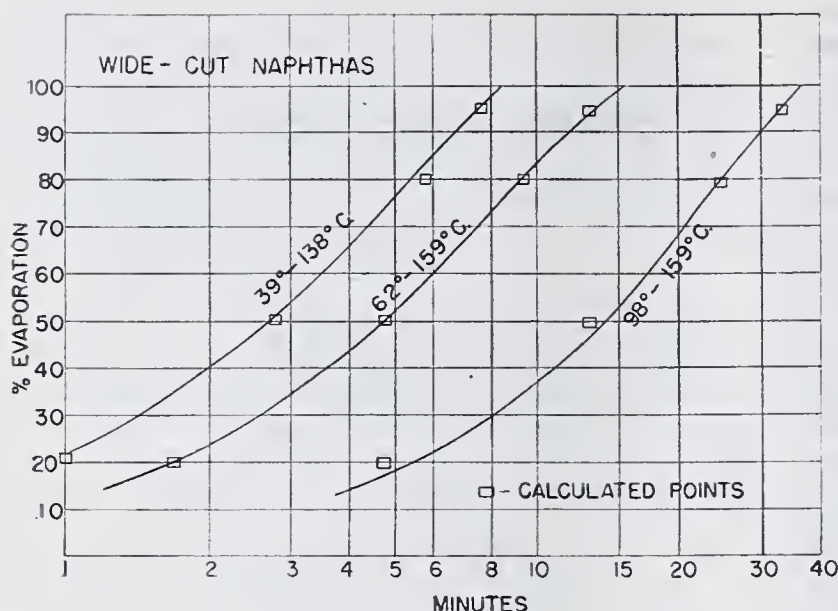


Figure 3

from the 50% distillation temperature. Conformity of the data with this correction is shown in Figure 3.

Evaporation indices for the 100° C. and 60° C.-wide naphthas may now be written as follows, for 20% evaporated:

$$I_{20} = 0.76 \text{ antilog } [0.01675(T - 13) - 1.22]$$

$$I_{20} = 0.76 \text{ antilog } [0.01675(T - 8) - 1.22]$$

Table II. Wide-Cut Petroleum Solvent Naphthas

	Wide-Cut Rubber Solvent	Wide-Cut Extraction Naphtha	Wide-Cut V.M. & P.
Gravity, ° A.P.I.	67.8	60.9	56.0
Sp. gr. at 60°/60° F.	0.710	0.735	0.755
A.S.T.M. distillation Initial B.P., ° C.	39	62	98
50% off	86	100	120
End point	138	159	159
Evaporation time at 25° C., minutes			
20%	0.9	1.7	5.5
50%	2.7	4.7	14
80%	5.4	9.2	24.6
95%	7.5	13.2	33

Table III. Latent Heats of Vaporization, Volume Basis

Boiling Point ° C.	Aro- matic	Latent Heats <sup>a</sup>		Specific Gravity at 60°/60° F.			Ratio of Differ- ences
		Straight- run	Differ- ence	Aro- matic	Straight- run	Differ- ence	
		Calories/cc.					
80	83	55.5	27.5	0.879	0.712	0.167	165
110	75.3	55	20.3	0.868	0.743	0.125	162
140	70	53.5	16.5	0.862	0.765	0.097	170
170	66.8	52.3	14.5	0.870	0.784	0.086	169

#### AROMATIC SOLVENTS

Because of their higher latent heat of vaporization, aromatic hydrocarbons evaporate more slowly at room temperature than paraffins of the same boiling range. The greatest difference is between benzene and an 80° C.-boiling paraffin—i.e., with increasing boiling point, or molecular weight, there is less difference between latent heats of aromatics and paraffins. These differences conform to differences in specific gravity, and the latter can be utilized to estimate evaporation rates of aromatic solvent naphthas from the data on paraffinic naphthas. Table III shows the ratios of these differences to be substantially constant for practical purposes.

In Table III, latent heats have been converted to a volume basis—since A.S.T.M. distillates are read by volume—by multiplying calories per gram at the boiling point by density. Density was not corrected to the same temperature as the boiling

point; but, for the purpose of these estimates, no gross error is hereby introduced, since (1) the divergence cubical expansion between aromatics and straight-petroleum naphthas is fairly small (6), and (2) the latent heats of vaporization of similarly boiling aromatics: paraffins roughly parallel each other between room temperature and their boiling points. Data for the aromatics were taken from the Doss compilation (5); the latent heats for the straight-run petroleum fractions from Fallon and Watson (7). Specific gravities of 40° C.-wide paraffinic naphthas were taken from Figure 4, which was plotted from data in Table I.

To utilize specific gravities,  $G$  and  $G_P$ , of aromatic solvents and paraffinic naphthas, respectively, as measures of latent heat, we take the following relation:

$$T_P = T + 50 (G - G_P)$$

where  $T_P$  is the 50% temperature of the equivalent paraffinic naphtha, and  $T$  is the actual 50% temperature of the aromatic. Thus 10° xylene evaporates at approximately the rate of a 40° C.-wide straight-run naphtha with a 50% distillation temperature of

$$137 + 50(0.862 - 0.765) = 142^\circ \text{ C.}$$

The general expressions for evaporation indices of solvents other than straight-run paraffinic types become:

$$I_{20} = 0.76 \text{ antilog } [0.01675\{T + 50 (G - G_P)\} - 1.22]$$

$$I_{50} = 0.27 \text{ antilog } [0.01675\{T + 50 (G - G_P)\} - 0.77]$$

$$I_{80} = 0.138 \text{ antilog } [0.01675\{T + 50 (G - G_P)\} - 0.48]$$

$$I_{95} = 0.105 \text{ antilog } [0.01675\{T + 50 (G - G_P)\} - 0.36]$$

Data in Table IV, which includes a number of commercial aromatic solvents, are plotted in Figure 5. Evaporation points estimated by the above method are also shown in Figure 5, are listed in Table V. It is important to note that these points are based on weight per cent evaporated. (The volume evaporometer used in this work evaporates 2.0 ml. of liquid, the families of evaporation curves here plotted are all on a volume basis. Most evaporometers operate on a weight basis.)

Concordance, on a weight basis, appears satisfactory in boiling ranges above toluene. It is noteworthy that 2° toluene and Solvesso No. 1 (a two-thirds aromatic naphtha with sim-

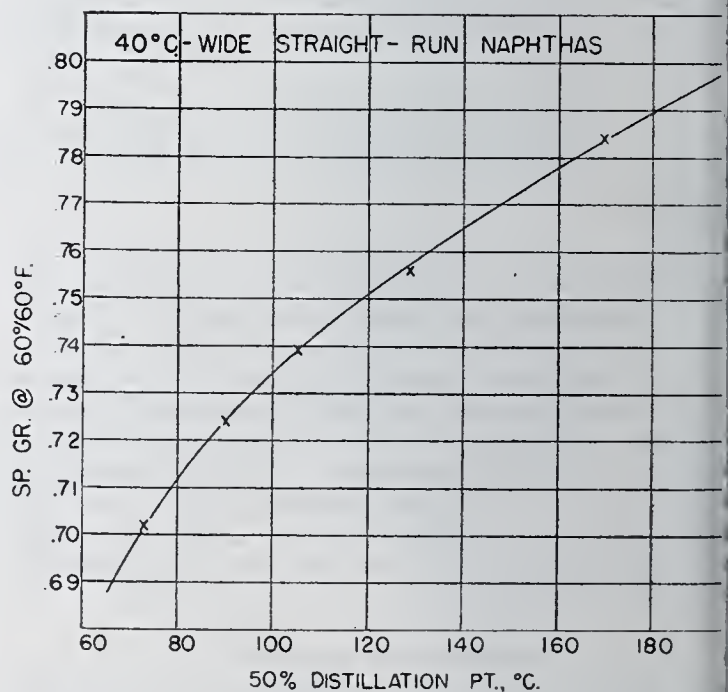


Figure 4



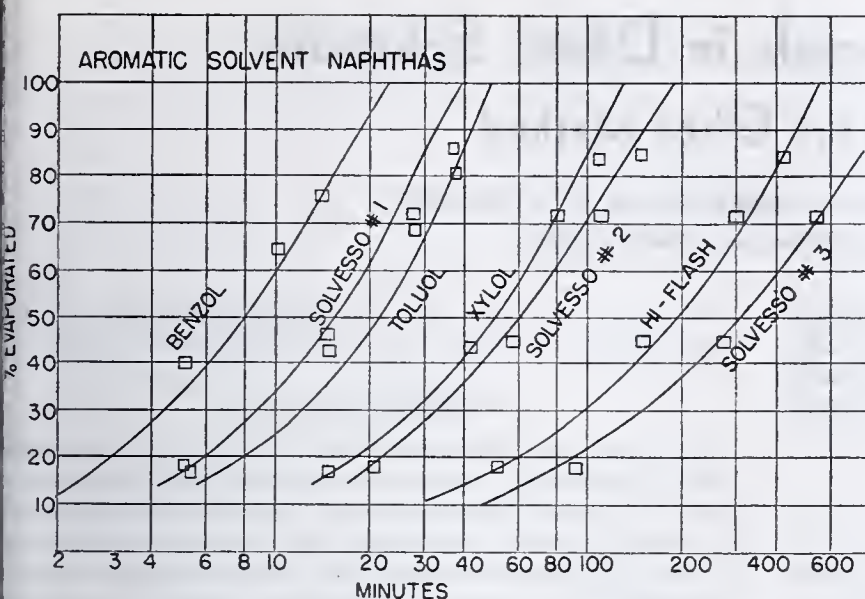


Figure 5

Table IV. Aromatic Solvent Naphthas

	2° Ben- zene	Sol- vesso 1	2° Tolu- ene	10° Xyl- ene	Sol- vesso 2	Coal- Tar Hi- Flash	Sol- vesso 3
Gravity, ° A.P.I.	29.5	39.3	31.6	32.6	33.6	31.1	29.5
Sp. gr. at 60°/60° F.	0.879	0.829	0.868	0.862	0.857	0.870	0.879
S.T.M. distillation							
Initial b.p., ° C.	78	99	108	134	137	152	174
50% off	80	111	110	137	147	171	187
End point	83	138	111	142	177	196	209
Vaporization time at 25° C., minutes							
20%	3	5.8	8	18	22	60	91
50%	8	15.5	21.5	51	60	193	310
80%	15.2	27.3	37	92	122	390	670
95%	21	36.0	46	120	170	504	...

Table V. Aromatic Solvent Naphthas

	Specific Gravity Difference	° C. Added to B.P.	Estimated Evaporation Points, by Weight			
Benzene	0.167	8.5	1.9 at 16.1%	5.2 at 40.4%	10.2 at 64.5%	14 at 76.5%
Solvesso 1	0.086	4.5	5.2 at 18%	15 at 45%	28 at 72%	39 at 85%
Toluene	0.125	6	5.5 at 17.1%	15 at 43%	28 at 69%	39 at 81%
Xylene	0.097	5	15 at 17.7%	42 at 44%	80 at 71%	110 at 84%
Solvesso 2	0.087	4.5	21 at 18%	58 at 45%	111 at 72%	150 at 85%
Hi-Flash	0.086	4.5	52 at 18%	155 at 45%	300 at 72%	410 at 85%
Solvesso 3	0.086	4.5	94 at 18%	275 at 45%	530 at 72%	...

0% distillation temperature) parallel each other in evaporation rate in both actual and estimated values; although agreement between these values for the individual solvent leaves something to be desired. Concordance in the case of benzene is poor, although the 76% point is close to actual. Benzene, however, is unique in a number of ways, and it evaporates much faster than other commercial aromatic solvents.

#### VARNISH SPIRITS

In the section above on "Evaporation Rate Curves", discussion of varnish spirits was postponed. The difference in specific gravity between the varnish spirits in Table I and the lower volatility mineral spirits—viz., 0.023—necessitates the use of the gravity correction, 50 ( $G - G_p$ ), and therefore there must be added 1° C. to its already 2° C. higher 50% distillation temperature.

In this connection it may appear, particularly in the case of the large number of mineral spirits commercially available, that an error in reading the 50% distillation temperature will bulk large in estimating evaporation rate. In such an event, the popular "average boiling point"—the arithmetic average of the 10, 20,

30, 70, 80, and 90% A.S.T.M. distillation temperatures—might be preferred to the 50% distillation temperature. Among the straight-run naphthas described in Tables I and II, however, in no case do the 50% points and average boiling points differ by as much as 2° C. Thus the 50% temperature,  $T$ , has been chosen for convenience.

#### POSSIBILITY OF WIDER APPLICATION

The present estimates are limited to commercially available solvent naphthas, or hydrocarbon thinners. It nevertheless appears possible to extend the method to include mixtures of hydrocarbons and oxygen-containing solvents, wherein latent heat of vaporization may vary rapidly with composition during the time of evaporation. Additional data covering volume changes upon mixing, together with evaporation rates of individual components, will then be required. Meanwhile, the present rough method is suggested as a substitute for routine control tests.

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#### Corrections

In the article on "Determination of Formaldehyde" [*IND. ENG. CHEM., ANAL. ED.*, **16**, 496 (1944)], reference 5 on page 496 should read: Boyd, M. J., and Bambach, Karl, *IND. ENG. CHEM., ANAL. ED.*, **15**, 314-15 (1943).

G. C. WHITNACK

In the note on "An Observation of Possible Value for Sugar Determinations" [*IND. ENG. CHEM., ANAL. ED.*, **16**, 537 (1944)], an error occurs in lines 15 and 33, where "grams" should read "milligrams".

The possibility of iodometric determination of the cuprous iodide has been discussed in detail by Shaffer and Hartman [*J. Biol. Chem.*, **45**, 365 (1920-21)].

DANIEL LUZON MORRIS



# Determining Phenols in Dilute Solutions

## Notes on the Gibbs Method

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Some suggestions concerning the performance of the Gibbs method are given, dealing especially with the removal of interfering substances and the best conditions for color development. The colors produced by certain phenol derivatives are listed, and an extraction method for the analysis of very dilute solutions is presented.

THE Gibbs method for determining phenols (1) has been used successfully in a number of laboratories, including that of The Dow Chemical Company. Nevertheless some workers have encountered difficulties; Buswell and Dunlop (4), for example, prefer to estimate phenol by means of its ultraviolet absorption. However, since the Gibbs procedure is capable of greater sensitivity and requires less expensive equipment, it will probably remain in common use. The present paper is intended to be supplementary to the procedure given in (1). The subjects discussed include notes on the distillation apparatus, interfering substances, best conditions for color development, and a procedure for determining phenol in more dilute solutions.

Although many substituted phenols produce colors in the Gibbs method, relatively few give the normal blue color of ordinary phenol and in those cases the sensitivity is usually less. Baylis (2) notes that *p*-cresol shows no color while *o*-cresol, like phenol, gives a blue. It is generally true that substitution in the para position reduces the sensitivity considerably, while ortho substitution with hydrocarbon groups has less effect and with halogen atoms shifts the color toward green. The colors produced by several phenols are shown in Table I, with concentration ranges which yield colors of about the proper intensity for comparison in 100-ml. Nessler tubes. All these compounds are volatile with steam and those which give colors with the Gibbs reagent will interfere in the phenol determination.

### DISTILLATION APPARATUS

In Figure 1 is shown a battery of six stills as they are set up in the Dow Analytical Laboratory, where numerous industrial waste and river water samples are run every day.

A Maharg all-glass distillation apparatus (mercury-sealed) is specified in (1). It is essential that the apparatus be thoroughly clean. Washing of the flasks and condensers, followed by steaming out with steam from pure water, is to be recommended between determinations, particularly when samples of varying concentration are being analyzed. Flasks with broken or chipped connecting tubes, which allow condensates to collect on the mercury seal, should be avoided.

Changing of receivers beneath the condenser is simplified by the use of an adapter shown in Figure 2. The adapter slides vertically on the condenser tube, cushioned by a rubber washer, A, which rests on bulge B in the tube. Glass ears at C support its weight when the volumetric flask, D, is in position. In building the apparatus, the portion of the condenser tip below E is first shaped and fitted with the washer. A piece of 1.9-cm. (0.75-inch) inside diameter tubing is drawn down to form the upper end of the adapter, and the condenser tip is inserted

from below. The lower end is next drawn out, the delivery tip is put on, then the condenser tip is sealed to the condenser at E.

### INTERFERING SUBSTANCES

In the analysis of industrial wastes, off-colors from the desired blue are sometimes obtained. Green-blues may indicate that part of the phenol contains halogen in the ortho position, possibly as a result of partial chlorination. They may also be produced by sulfides, which according to the amount present can yield off-shades varying through yellow-greens to pink. Williams (9) suggested removing sulfides with lead oxide or carbonate, while Renzoni (6) recommended the use of freshly precipitated cadmium carbonate. The authors have used copper sulfate, adding a small volume of strong copper sulfate solution to the measured sample and filtering into the distilling flask.

To prevent interference from volatile acids such as salicylic in industrial wastes, double distillation may be necessary. The first distillation is made as usual (see 1.6, p. 246, 1), though sulfuric acid may be substituted for phosphoric if aniline or other weak bases are present. The entire 500 ml. of distillate are then returned to the distillation flask after the latter has been washed

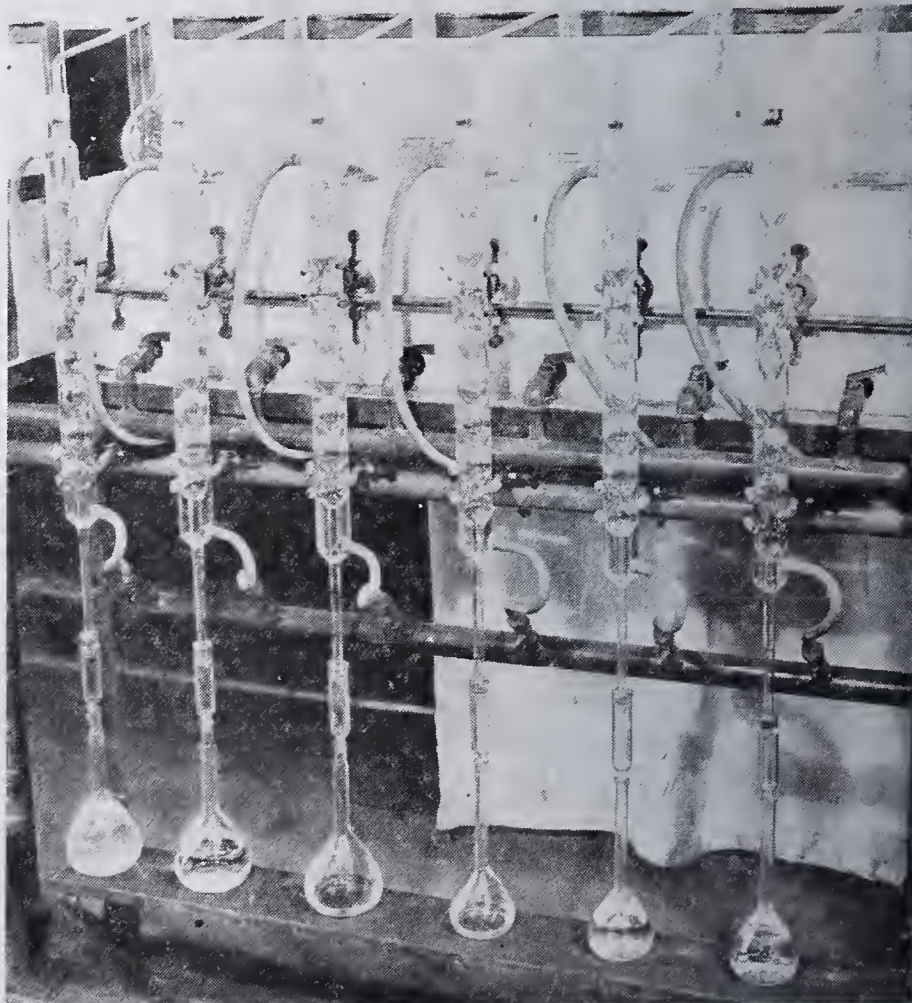


Figure 1. Battery of Six Stills



grams of fairly coarse marble chips are added, and the second distillation is carried out. Calcium carbonate furnishes sufficient alkalinity to retain salicylic acid, but not enough to interfere with the distillation of phenol.

#### COLOR DEVELOPMENT AND COMPARISON

Difficulties in the method may frequently be traced to the instability of 2,6-dibromoquinonechloroimide. It has been found advisable to purchase this compound in brown glass bottles containing only 1 gram each. These are kept closed until needed; after a bottle is opened the material in it has occasionally been found to decompose within a week or two. The precautions given in (1, p. 48), concerning the preparation and use of solutions of this reagent, should be carefully observed. The authors employ the alcoholic solution (1.61) exclusively.

Since the dye produced by reaction of the reagent with phenol is an oxidation-reduction indicator as well as an acid-base indicator (5), it is essential that the pH and the oxidation potential be maintained within the specified limits. It is a good practice to add copper sulfate solution to all samples and standards (1).

Solutions in the Nessler tubes will turn pink if exposed to sunlight after the addition of reagent. For the best results it is necessary to keep the tubes in the dark during color development. This is conveniently done by keeping them in a box similar to the one illustrated in Figure 3. After 4 hours or longer, the comparison is made in a Fisher Nessler tube support or similar rack, by fluorescent light.

The reaction between phenols and 2,6-dibromoquinonechloroimide proceeds slowly and the color continues to develop for a long period. On this account the samples and standard should be prepared at the same time. In any attempt to determine phenols with a photoelectric colorimeter (8), the standard curve should be prepared with allowance of a definite period for color development at a specified temperature. The same conditions should be adhered to carefully in the analysis of a sample.

#### ANALYSIS OF MORE DILUTE SOLUTIONS

Various methods for concentrating dilute phenol solutions have been proposed, involving evaporation of an alkaline solution (7), distillation (3), or extraction of the free phenol with ether (6). A procedure based on developing the color in a larger volume of sample and subsequently extracting the colored compound can also be used. This procedure multiplies the sensitivity of the Gibbs method by about 10 and increases the stability of the color.

The reagents are the same as for the usual Gibbs method, with the further inclusion of 1 to 4 hydrochloric acid, chloroform, methanol, or Formula 30 alcohol, and approximately 0.1 *N* alcoholic sodium or potassium hydroxide. More than ordinary care must be taken to obtain phenol-free water for the standards. Add about 200 ml. of finely powdered activated carbon to 37.85 liters (10 gallons) of a good grade of distilled water and agitate with an air stream for 30 minutes. To the suspension add 0.4 gram of aluminum sulfate and 2 grams of sodium bicarbonate, agitate for 15 minutes longer, allow to settle, and filter off as needed.

In the absence of substances which interfere in the ordinary Gibbs method, 1 liter of the neutral sample may be run directly; otherwise a single or double distillation is necessary. A liter of the water is distilled and the entire amount of phenol is considered to be recovered when 900 ml. have been collected in the first distillation, or 850 ml. in the second. Standards containing 0,

0.5, 1.0, 2.0, 3.0, 5.0, 7.0, and 10 parts per billion are prepared from standard phenol solution 1.8 (1, p. 247), and phenol-free water. These should have the same volume as the sample. One milliliter of the standard phenol solution diluted to 1 liter corresponds to 1 part of phenol per billion. To each sample and standard add 20 ml. of alkaline borate buffer solution, 10 ml. of copper sulfate solution, and 3 ml. of the freshly diluted alcoholic 2,6-dibromoquinonechloroimide reagent. Mix well and allow to stand in a dark place overnight.

Transfer to a large separatory funnel, add 5 ml. of 1 to 4 hydrochloric acid and 18 ml. of chloroform, mix well, allow to separate, and draw off the chloroform layer into a 50-ml. Nessler tube. Extract the solution again with 10 ml. of chloroform and add to the same tube. To each tube add 20 ml. of methanol or Formula 30 alcohol and 0.10 ml. of 0.1 *N* alcoholic sodium or potassium hydroxide, invert a few times, and compare the colors.

It is barely possible to detect 0.25 part of phenol per billion, provided that interferences are absent and the standard water is pure. The detection of 0.5 part is rather easy with phenol, which gives a blue-green color. The blank may be slightly yellow because of the presence of excess reagent, the intensity of the yellow color being greater with older reagent. With *o*-chloro-

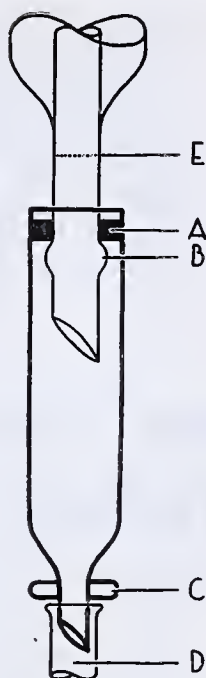


Figure 2. Adapter

Table I. Colors of Several Phenols in the Gibbs Test

Phenol	Color	Concentration Range, Parts per Billion
Ordinary	Blue	5-100
<i>p</i> -Chloro	Blue	20-400
<i>p</i> -Bromo	Blue	25-500
<i>o</i> -Chloro	Green-blue	10-150
<i>o</i> -Bromo	Green-blue	10-200
2,4-Dichloro	Green-blue	40-800
2,5-Dichloro	Green-blue	30-600
2,6-Dichloro	Green	30-600
Trichloro	Insensitve	..
Tribromo	Insensitve	..
<i>o</i> -Phenyl	Blue	10-200
2-Chloro-6-phenyl	Blue	10-200
4-Chloro-6-phenyl	Green-blue	10-200
<i>p</i> -Phenyl	Insensitve	..
<i>p</i> -tert-Butyl	Insensitve	..

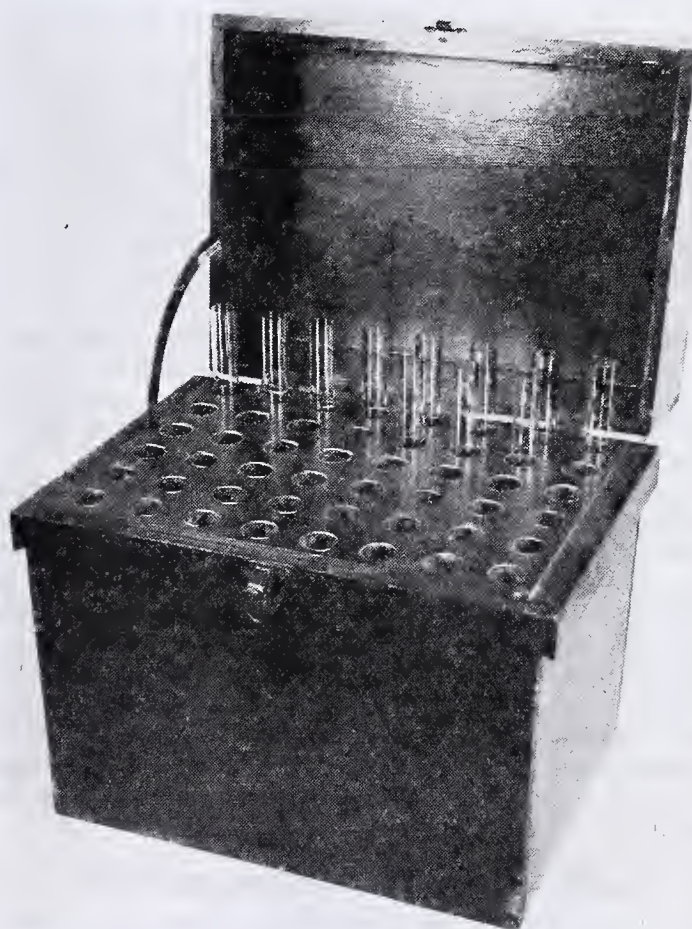


Figure 3. Tube Holder



phenol the color is much the same as with phenol, but the test is only about one half as sensitive. The color from *p*-chlorophenol is more toward the blue, and the sensitivity is only about one fourth that of phenol.

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## Determination of the Nutritive Value of the Proteins of Food Products

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The nutritive value of food protein cannot be accurately assessed from a determination of the amino acid content, nor from animal feeding experiments that fail to credit the protein with all its functions in the body. A study of the nitrogen economy of the animal when fed the protein to be tested under certain essential conditions is capable of giving a complete and reasonably satisfactory picture of protein utilization in the body, in digestion as well as in metabolism. The net protein value combines all this information in one figure. Illustrations of application of the method to problems of the storage and processing of foods are given.

THE protein content of food products has an uncertain significance for their evaluation in nutrition, because proteins vary widely in the extent of their digestion in the alimentary canal, and even more so in the extent to which the end products of their digestion, largely amino acids, are available for those functions in the body peculiar to dietary protein. These functions are bewildering in their complexity and in their involvement in life processes, but quantitatively the construction of new protein material in growth and the maintenance of the nitrogenous integrity of the tissues already formed are by far the most important.

The differences that exist in the value to the animal body of the proteins in food products, the extent to which food proteins supplement each other's amino acid deficiencies when combined into diets, particularly the best method of supplementing the proteins of white flour, and the effect of storage and commercial processing on the protein value of food products in nutrition are all problems of importance to food technologists. How can these problems be most effectively tackled in the chemical or the biochemical laboratory?

At the present time, three methods are being used for these purposes: (a) determination of the amino acid contents of foods or food proteins, (b) measurement of the ratio of gain in weight to protein intake of growing rats subsisting on rations in which the protein content is the only factor limiting growth, and (c) measurement of the gain in nitrogen to the bodies of growing rats resulting from the consumption of diets complete in all respects but protein. The latter method can be extended to mature, pregnant, or lactating animals, and to the human subject.

This paper considers briefly the advantages and disadvantages of each method for the purposes for which they are being employed.

#### AMINO ACID ANALYSIS OF PROTEINS AND FOODS

The basis upon which this method rests is that the nutritive quality of food proteins is determined entirely, or largely, by their content of amino acids, particularly of those nine or ten

amino acids commonly classed as dietary essentials on the evidence of Rose's well-known experiments on growing rats. Undoubtedly the content of a food in those amino acids that the body cannot synthesize from ordinary dietary components set an upper limit to its usefulness in serving the biological function of dietary protein; and to this extent the basis is sound.

The amino acid analysis of the proteins in a food product by chemical methods is laborious and the various analyses for individual amino acids are not of equal precision; those for leucine and valine in particular leave much to be desired (4). The microbiological methods suggested for determining the amino acid contents of food products are still in the experimental stage. However, it is not too much to hope that in the near future satisfactory methods, chemical or microbiological in nature, will be available for all the amino acids commonly believed to be dietary essentials. It should be emphasized, however, that satisfactory analyses for all these essential amino acids must be at hand before any one food product can be evaluated; otherwise, the possibility exists that the amino acid, or acids, for which no analysis is available may be, or include, the amino acid limiting the nutritive value of the contained proteins.

When the ultimate goal of amino acid analysis of food protein is attained, it will be possible from the data secured on many foods to predict which are the best in supplying the needs of the body for the essential amino acids, in so far as these needs are known, and in particular to predict which protein mixtures in individual foods will correct best each other's deficiencies in essential amino acids. But these predictions cannot be expressed quantitatively nor can they be made with any great assurance because the chemical picture of food proteins is inevitably altered and distorted by biological factors before a true picture of nutritional quality is secured.

The disturbing biological factors that impair the usefulness in practical nutrition of a knowledge of the amino acid contents of foods are as follows:

The digestibility of food proteins in the alimentary tract is largely independent of amino acid composition, and may be determined, as Mendel and Fine showed many years ago (15-19) by the constituents of foods other than protein. The digestive apparatus of the animal, though remarkably efficient under the most favorable conditions in reducing dietary protein to its ultimate building stones, may be impeded and frustrated in its operation by those nonprotein constituents of food that successfully resist its attack (the cellulosic and hemicellulosic components in particular), while preventing complete access of the proteolytic enzymes to their proper substrates.

The indigestible residue of food protein may not be representative in its amino acid constitution of the food from which it was derived. This may be inferred from the known differences in the ease with which different peptide linkages are split by the digestive enzymes. An instance in point was reported by Jones and



Waterman (13) in their study of the digestion in vitro of arachin, the chief protein of the peanut.

The amino acids are liberated from food protein in digestion, not evenly, but at different rates characteristic of the amino acid, or of the manner of its linkage in the protein molecule (12, 19). They are also absorbed into the blood at different rates (5). The fate of the end products of protein digestion in metabolism is not determined solely by their suitability in meeting the needs of the tissues for anabolic purposes. The tissues are also continually in need of energy in the performance of physiological work. The organic nutrients serve interchangeably in the metabolic mixture from which this energy is extracted. Amino acids resulting from protein digestion are thus inevitably drawn into the oxidative reactions of the body in proportion to their relative concentrations in the influx to the tissues of the end products of digestion (21, 24), and probably also in proportion to their relative susceptibility to oxidation with reference to each other and to that of sugars and lipids.

In view of the factors listed above, it would be remarkable indeed if the mixture of amino acids that is ultimately available to the tissues for synthesis of proteins and other nitrogenous tissue constituents were closely similar to the mixture of amino acids present in the food as consumed. Deficiencies in the latter could of course persist through all these vicissitudes, but excesses of certain amino acids may be wiped out, while proportions of others that were originally adequate may develop into deficiencies.

It seems fair to conclude that the amino acid analysis of food proteins may yield information of great significance concerning their nutritive values and their interrelationships in the diet, but that such an analysis cannot take the place of a biological assay. It is doubtful that an amino acid analysis of foods can measure, or explain, the changes in nutritive value that occur during storage and heat processing, especially an improvement in nutritive value such as many legume proteins undergo on heat treatment.

#### GROWTH-PROMOTING VALUE OF FOOD PROTEINS

Of the biological assays of food proteins in current use, the most popular undoubtedly is the determination with young albino rats of the ratio of gain in body weight to protein consumed during a 4- to 6-week period on diets containing variable concentrations of protein such that, ordinarily, the concentration used is inadequate to promote maximal growth, being otherwise complete. The method is a simplification of one proposed 25 years ago by Osborne, Mendel, and Ferry (33). The original method proposed that food proteins be compared by determining the maximum ratio of gain in weight to protein consumed among ratios secured by varying the concentration of protein in the experimental diet. This maximum ratio for casein was 2.25 at a protein level of 12%, and for lactalbumin, 3.01 at a protein level of 7.9%.

The simplified method in common use has served a useful purpose in comparisons of protein "quality" in the biological sense among many food products studied. For many purposes, it is probably satisfactory, though its most appealing characteristic is its simplicity. Requiring no control of the food intake of the experimental animals and no other measurements than the periodical taking of body weights, it seems to be an ideal technique when comparisons of large numbers of food products are to be made.

The method was subjected to critical scrutiny in a paper published by the author 20 years ago (23). In the present connection it may be well to restate the implications of the method, but to discuss them mainly in the light of information revealed since the publication of this review.

In crediting dietary protein only with the growth induced in experimental animals and in permitting an unlimited consumption of food, the method implies that there is no requirement of protein for the mere maintenance of life, since only on such an assumption would it be expected that a constant ratio of gain in weight to protein eaten would be obtained regardless of the intake of protein. The reality of this implication would be difficult to defend at the present time, especially in view of Osborne

and Mendel's determinations (32) of maintenance requirements for various wheat proteins and the more extensive work of Smuts (35). If the implication is wrong, one would expect the ratio of gain in weight to protein consumed to increase on the same diet as the intake of protein (food) increases, since with the greater intake of protein, a greater proportion of it would be available for growth. Hoagland and Snider (9) found this to be true in that male rats, consuming larger amounts of food than female rats and consequently growing faster, exhibited almost always the larger ratio of gain to protein eaten. Stewart and associates (36) found that a restriction of the food intake of rats lowered considerably the ratio of gain to protein eaten for the same protein source. Thus, for rolled oats the ratio averaged 1.52 under conditions of *ad libitum* feeding, but only 1.14 when the protein (food) consumption was restricted by about 40%. Of the same significance is the fact that the ratio was almost twice as variable with unrestricted feeding as with equalized feeding, the standard deviations being, respectively, 0.167 and 0.094.

The method of measuring protein quality by an efficiency ratio of growth to protein eaten implies that the protein content of the gains in body weight of growing animals is constant regardless of the age or size of animal, the quality of the protein, or the rate of growth. To the extent that the gains differ in their content of protein, fat, and water they do not represent equal nutritive effects, and hence are not comparable. For example, it would be expected that a gram of dietary protein would induce a greater gain in body weight of an animal if this gain contained 15% of protein than if it contained 20%, assuming that other growth essentials are present in adequate amounts. In scientific investigations with farm animals it is generally recognized that live weight increase is not a reliable measure of nutritive effect, a point discussed by Armsby (2, pp. 196-9) as early as 1917. Since then evidence of the variable composition of live weight gains has been presented by Mitchell and Carman (26) for rats, and by Fraps and Carlyle (7) for chickens. Hamilton (8) has shown that the live weight gains of growing rats, when put on at the same rate, may vary in their content of protein and energy with varying levels of the same dietary protein, while Kik (14) proved that two proteins differing in nutritive value and fed in such amounts to growing rats receiving equal amounts of total food as to induce equal gains in body weight, may produce different proportions of protein in the body weight gains, the better protein producing the higher content of this nutrient in the gains secured. There is a distinct tendency for the more rapid gains in body weight to have the greater content of fat and the smaller content of protein, as Mitchell (22) has demonstrated for cattle and Ellis and Zeller (6) for pigs.

The disturbing effect of a variable composition of body weight gains in the interpretation of a protein nutrition experiment is well illustrated by an experiment reported from the author's laboratory (3) concerned with the comparative nutritive value of the protein of milk curd and of the cheeses produced from it by various types of ripening. When fed in equal amounts in paired-feeding tests, the protein of Limburger cheese was found to be equal to that of fresh milk curd in growth-promoting value, although it was definitely less digestible and possessed a lower biological value. The explanation lay in the composition of the body weight gains: the gains put on by the Limburger cheese ration were definitely lower in protein content and higher in their fat content, than the gains produced on the milk-curd ration. In the same sort of tests, Swiss cheese protein proved superior to milk curd in growth-promoting value, though no better in biological value and definitely inferior in digestibility. An analogous difference in the composition of body weight gains between the cheese ration and the milk-curd ration must have occurred.

Thus, the two clear implications of this method for the biological assay of protein quality in nutrition are untenable. Some evidence of the seriousness of these basic errors in producing variation in the ratio of body weight gain to protein intake has been given above. The situation may be further pictured by the hypothetical illustrations given in Table I, showing to what ex-



**Table I. Effect of Increasing Intake of Same Diet on Ratio of Body Weight Gain to Protein Intake**

	Case 1	Case 2	Case 3	Case 4	Case 5
Weight of rat, grams	50	50	50	50	50
Daily food, grams	4	5	6	7	8
Daily protein, mg.	400	500	600	700	800
Protein for maintenance, mg.	200	200	200	200	200
Protein for growth, mg.	200	300	400	500	600
Net protein for growth, mg.	110	165	220	275	330
Protein content of gain, %	23	22	21	20	19
Daily gain, grams	0.48	0.75	1.05	1.37	1.74
Gain per gram of protein eaten, grams	1.20	1.50	1.75	1.96	2.17

tent the ratio may be changed by changing food intake, although the utilization of the dietary protein remains unaltered.

In each of the five illustrations, the weight of rat is the same, but the daily consumption of food increases from 4 to 8 grams. The diet in all cases is the same and contains 10% of whole wheat protein. The protein requirement for maintenance for this protein was found by Osborne and Mendel (32) to approximate 3 mg. per gram of rat per day. This requirement has been raised to 4 mg. because at a 10% level in the diet, utilization in metabolism is less (21, 24). The wheat protein in all cases is assumed to be 85% digestible and to have a biological value of 65% (27), the "net protein" thus amounting to 55% ( $0.85 \times 0.65 = 0.55$ ) of the total protein. The protein content of the body weight gains is assumed to decrease from 23 to 19% as the food intake and the rate of growth increase, in conformance with evidence cited above.

These assumptions seem reasonable and to a large extent are based upon experimental evidence. They lead to the expectation that the ratio of body weight gain to protein eaten may be changed, for a whole wheat diet containing 10% of protein, from 1.20 to 2.17 by merely increasing the intake of food. This demonstration accounts for the large experimental error to which the ratio is subjected under conditions of *ad libitum* feeding. It also indicates clearly that this method of assaying protein quality will definitely tend to exaggerate differences in this respect among different protein foods, because the rations containing the better protein foods will generally be consumed in the larger amounts and will thus be given an undue advantage over the poorer protein foods. Equating the food intakes of rats on comparable diets will overcome this effect, but the uncertainty concerning the protein content of body weight gains is inherent in the method.

In brief, this method of assaying food protein biologically may be expected to exaggerate quality differences among proteins when these are considerable, and to obscure them when they are inconsiderable, on account of the large experimental error to which the method is subject as it is ordinarily employed.

#### BIOLOGICAL ASSAY OF FOOD PROTEINS BY MEANS OF NITROGEN METABOLISM STUDIES

This method was first introduced by Thomas (37) in 1909, and was later adapted to the growing rat by Mitchell (20). Many modifications in the method have since been made (25, 28). It is based upon the fact that protein is the only considerable source of dietary nitrogen and that the disposition of protein within the body of an animal can be followed with considerable accuracy by determining the intake and fecal and urinary excretion of nitrogen. When this is done under carefully selected and controlled conditions, it is possible to compute from the data secured (a) the digestibility of the protein, (b) its utilization in metabolism for all purposes, expressed as a percentage called by Thomas the "biological value", and (c) the "net protein content" of the food assayed, equal to the total content times the digestion coefficient (expressed as a decimal) times the biological value (also expressed as a decimal).

Unlike the biological assay method previously considered, this method credits the dietary protein with all its characteristic functions in the body, maintenance as well as growth in the growing rat, and it directly deter-

mines the storage of protein in growth rather than assumes that this storage is proportional to body weight gains. Furthermore its accuracy is such (25) that it can detect differences in digestibility and biological value of proteins of a magnitude of two or three percentage units, using a battery of only 10 animals.

An illustration of its value in detecting changes in the nutritive value of a food protein (soybean proteins) during storage is afforded by the data summarized in Table II.

The soybeans used in this test were of the Illini variety and the 1942 crop. They were stored at a temperature of 78° to 80° F. in air-tight containers, either whole or as a ground defatted meal that had been heated in the autoclave for 1.5 hours at 17 pound steam pressure. After 8.5 months, and again after 12 months storage, containers of both whole beans and autoclaved meal were opened and tested for protein quality by the nitrogen balance method. The whole beans before testing were ground, extracted with ether, and heated for 1.5 hours in the autoclave at 17 pounds' pressure—i.e., the same treatment to which the meal had been subjected prior to storage.

The results indicate that after 8.5 months' storage, the protein in the beans stored whole with no pretreatment had suffered a marked decrease in digestibility, averaging 7 percentage units and an even more marked decrease in biological value, averaging 11 percentage units, as compared with the beans ground and heated prior to storage. Both differences were statistically significant. At the end of 12 months' storage, the difference in digestibility between the two products was still distinct, but a clear difference in biological value was not demonstrated, possibly because of two erratic biological values secured for the ground meal. The results confirm and extend those reported by Jones and Gersdorff (11), who reported a drop in the digestibility *in vitro* of the proteins of soybeans on storage.

The results of a similar test on corn proteins are summarized in Table III. In this test the corn was stored either whole or ground, but with no preheating. The method of storing evidently had no effect, either on the digestibility or the biological value of the corn proteins, but storage for 8 months depressed equally the biological value of the proteins in corn stored whole or ground, while leaving the digestibility unaffected. From this outcome, one might conclude that, in the soybean test, it was the preheating, rather than the grinding, that exerted the stabilizing effect on the proteins during storage. The results on corn do not harmonize entirely with those reported by Jones, Divine, and Gersdorff (10).

#### NET PROTEIN VALUE OF FOOD PRODUCTS

While the nitrogen balance method for the biological assay of protein quality distinguishes between losses of nitrogen in the digestive and in the metabolic processes of the animal body, there are distinct advantages in securing in a single figure an appraisal of the value of a food as a source of dietary protein. Such a figure should involve not only the biological value of the protein and its digestibility, but also its original content of "conventional"

**Table II. Changes in Digestibility and Biological Value of Proteins of Soybeans during Storage**

(All determinations made on beans after autoclaving)									
Soybeans Stored 8.5 Months					Soybeans Stored 12 Months				
Rat No.	Whole, Unheated		Ground, Autoclaved		Rat No.	Whole, Unheated		Ground, Autoclaved	
	True digestibility %	Biological value %	True digestibility %	Biological value %		True digestibility %	Biological value %	True digestibility %	Biological value %
292	77	65	85	80	337	78	60	85	71
295	78	61	82	71	340	79	67	85	71
298	76	61	86	73	343	80	67	82	82
301	77	61	84	70	346	77	70	85	67
304	84	61	85	71	349	81	66	83	52
293	82	64	88	72	338	79	78	85	73
296	79	65	85	74	341	81	68	84	67
299	75	59	84	71	344	79	62	86	68
302	76	60	87	76	347	81	60	84	76
305	79	69	86	79	350	79	63	82	67
Av.	78.3	62.6	85.3	73.7		79.4	66.1	84.0	68.2



protein—i.e.,  $N \times 6.25$ . Such a value was proposed by the author and Carman (27) many years ago, and has been called the "net protein value" of the food which may be computed on the dry basis or on the fresh basis, as in the tabulation below:

Food	Total Protein %	Digestibility of Protein %	Digestible Protein %	Biological Value %	Net Protein %
Eggs	13.4	100	13.4	83	12.5
Lean ham	19.8	100	19.8	74	14.6
Wheat	12.5	91	11.4	67	7.6
Soybeans	32.8	84	27.6	72	19.9
Soybeans, stored for 8.5 months	32.8	78	25.6	63	16.1

The net protein values as thus computed are mainly of comparative significance, because of the variation to which biological values are subject as the level of protein in the diet changes.

#### CRITIQUE OF THE NITROGEN BALANCE METHOD OF ASSAY OF FOOD PROTEIN

Measurements of protein digestibility and of the biological value of the absorbed protein must, in the present state of biochemical technique, be expressed in terms of nitrogen. This is not the most satisfactory situation, because all food nitrogen is not in the form of protein, or amino acids, or amino acid derivatives, nor is the nitrogen of the tissues present there only in such compounds as these. However, the ambiguity introduced into measurements of protein digestibility and biological value by employing nitrogen as a conventional equivalent of protein is not generally large. Certainly no substitute procedure can claim to be as satisfactory. Perhaps a more serious objection to this emphasis upon protein nitrogen is that it neglects the function of dietary protein of providing the body with comparatively large amounts of readily available phosphorus, which is not an integral part of the amino acid structure.

The nitrogen balance method of assessing protein quality was proposed by Thomas at a time when Folin's theory of protein metabolism, with its sharp distinction between exogenous and endogenous metabolism, was commonly accepted. In assuming a constant (biologically speaking) erosion of nitrogenous material from the animal body, commensurate with the maintenance requirement of protein, the method tacitly assumes the reality of Folin's endogenous catabolism. However, the work of Schoenheimer and his associates on amino acid metabolism using the nitrogen isotope may seem to cast doubt upon any assumption of this nature. These revolutionary investigations swept away all sharp distinctions between the endogenous and the exogenous metabolism that form the basis of Folin's theory of protein metabolism. They afford a convincing picture of the dynamic equilibrium existing between the tissue proteins and the amino acids of dietary origin in the circulating fluids of the body. However, they do not change the significance of the end results of protein metabolism as revealed by such a nitrogen balance study as would be undertaken in the course of securing digestion coefficients and biological values according to the Thomas method or some modification of it. The nitrogen in the urine on a nitrogen-free diet still represents a minimum or basal level of nitrogen loss from the tissues, bearing a relationship to the basal metabolism of energy (35). Considerable circumstantial evidence can be adduced to the effect that this basal rate of nitrogen output in the urine continues at a constant level, biologically speaking, during regimes of adequate protein nutrition.

One of the most convincing bits of evidence of this description may be found in a publication by Ackerson and Blish (1) on the utilization of the protein of corn by hens. Nonmolting hens excreted an average of 143 mg. of nitrogen per kg. of body weight per day on a nitrogen-free diet, while molting hens on the same diet excreted 217 mg. However, assuming that these greatly different values for the constant catabolism of nitrogen persisted during periods of feeding a corn diet, the same average biological

value of 68 was obtained for a group of 19 nonmolting birds and for a group of 8 molting birds.

The urinary nitrogen during periods when test proteins are being fed consists definitely of two fractions, one the constant contribution from the tissues, and the other related to the level of protein feeding and the efficiency with which the absorbed amino acids satisfy the prevailing requirements of amino acids by the tissues. This fraction is partly of tissue origin and partly of dietary origin, because of the dynamic relation existing between tissue proteins and dietary amino acids. But this dynamic relationship is not anarchistic in character. Through the welter of reactions in which the tissue proteins are involved, the cells and organs of the adult body retain their original form and size and the chemical structures of the protein molecules emerge unchanged, as Schoenheimer and Rittenberg admit (34). Discussing the many interconversion reactions divulged by the isotope method, Moss and Schoenheimer (30) believe that they cannot be regarded "merely as steps in metabolic degradation. They seem rather to represent automatic and noninterruptable biochemical processes, of synthesis as well as degradation, which are balanced by an unknown regulatory mechanism so that the total amount of the body material and its composition do not change."

Table III. Changes in Digestibility and Biological Value of Protein (Nitrogen) of Corn (U. S. Hybrid 13) during Storage

Rat No.	Before Storage		Rat No.	After Storage for 8 Months			
	True digestibility	Biological value		Stored True digestibility	Whole Biological value	Stored True digestibility	Ground Biological value
	%	%		%	%	%	%
259	90	72	322	91	53	92	60
261	91	62	325	92	54	92	60
263	93	69	328	93	57	94	60
265	94	68	331	91	56	91	55
267	93	69	334	89	52	90	62
260	90	62	323	92	60	91	60
262	89	61	326	93	67	90	59
264	95	55	329	93	58	91	54
266	92	62	332	92	61	91	56
268	96	69	335	91	59	91	60
Av.	92.3	64.9		91.7	57.7	91.3	58.6

The "exogenous nitrogen" of the urine, to use the obsolete term of Folin, is thus both of tissue and of diet origin, but the rate of its excretion can nevertheless be assumed to depend solely upon the level of protein intake and upon the extent to which the dietary protein is used in the replacement of tissue losses of nitrogen in the adult animal. In the growing animal the "exogenous nitrogen" of the urine will be reduced to the extent that dietary amino acids are used in the elaboration of new tissue in growth. To all intents and purposes, therefore, this fraction of the urine nitrogen measures the wastage of dietary nitrogen in metabolism under the conditions of diet control imposed in a well-considered determination of the biological value of protein.

The essential accuracy of the assumptions upon which this determination is based has been tested in the author's laboratory by comparison with a method of measuring protein quality that does not involve these assumptions. The results obtained in tests upon two protein sources were found to agree satisfactorily (25). The reproducibility of results secured at different times upon the same protein source is also satisfactory as the method is used in this laboratory, although very occasionally unaccountable erratic values, such as three of those listed in Table II, are obtained. Any method of biological assay of food products may thus go "haywire" on occasion.

#### NITROGEN REPLACEMENT VALUES

Murlin and his associates (31) have introduced a new method of interpreting nitrogen balance data relating to the utilization



Table IV. Replacement Values of Protein of Exploded Wheat<sup>a</sup> on Proteins of Unprocessed Wheat<sup>b</sup>

Period	Pair No.	Rat No.	Daily Nitrogen Intake			Daily Nitrogen Balance			Re- place- ment Values %
			Ex- ploded wheat	Unproc- essed wheat	Aver- age	Ex- ploded wheat	Unproc- essed wheat	Differ- ences	
			Mg.	Mg.	Mg.	Mg.	Mg.	Mg.	
I, 5% protein in diets	1	1	150	...	...	-3.5	17.3	20.8	86
		2	...	159	155	...	...	...	...
	2	3	134	...	...	-8.9	...	...	...
		4	...	142	138	...	9.3	18.2	87
	3	5	134	...	...	15.4	...	...	...
		6	...	142	138	...	5.4	-10.0	107
	4	7	134	...	...	-5.8	...	...	...
		8	...	142	138	...	12.1	17.9	87
	5	9	134	...	...	-14.4	...	...	...
		10	...	142	138	...	5.9	20.3	85
II, 4% protein in diets	1	1	...	87	...	...	-15.8	...	...
		2	...	88	87	-26.3	...	10.5	88
	2	3	...	78	...	...	-17.0	...	...
		4	...	79	78	-34.8	...	17.8	77
	3	5	...	61	...	...	-25.5	...	...
		6	...	62	61	-34.6	...	9.1	86
	4	7	...	89	...	...	-7.9	...	...
		8	...	90	89	-31.5	...	23.6	74
	5	9	...	95	...	...	-18.2	...	...
		10	...	96	95	-29.1	...	10.9	89
• Av.									86.8

<sup>a</sup> Av.<sup>a</sup> "Puffed Wheat", Quaker Oats Co.<sup>b</sup> Test carried out with aid of funds from General Mills, Inc.

of protein in nutrition that possesses the advantage of removing the need of a standardizing period for the determination of the body's contribution to the fecal and urinary nitrogen. It was devised especially for use in experiments on human subjects, for whom low-protein diets are extremely unpalatable. The method simply compares the nitrogen balances of adult experimental subjects on the same intake of nitrogen from the food under test and in another period, from a reference protein of high nutritive value, such as the protein of milk or of egg.

The method may be applied to such a problem as that of the injury to food protein brought about by heat processing. In Table IV, the method has been used to assess the extent of heat injury induced in the whole wheat kernel by subjecting it to the "gun explosion" method of preparing a breakfast food. Five pairs of adult rats have been carried through two nitrogen metabolism periods, one rat in each pair receiving its protein from processed wheat and the other rat in equal amounts from unprocessed wheat. In the second metabolism period the sources of protein for the paired rats were reversed. The replacement values given in the last column of the table indicate the extent of heat injury and were computed by the formula

$$R.V. = 100 - \left[ \frac{B_1 - B_2}{I} \times 100 \right]$$

in which  $B_1$  is the nitrogen balance of the rat subsisting on the unprocessed wheat diet,  $B_2$  is the nitrogen balance of its pair mate subsisting on the processed wheat diet, and  $I$  is the average of the nitrogen intakes of the two rats in the pair, which should be practically the same.

The average replacement value of 86.8 shows that the processing of wheat by the gun explosion method has impaired the protein value in adult rodent nutrition by 13.2%. For growing animals, the impairment may be greater than this. It is interesting to note that, with adult human subjects, Murlin, Nasset, and Marsh (31) report egg replacement values of 70 and 57 for two whole wheat cereals (Ralston's Wheat Cereal and Puffed Wheat) subjected, respectively, to mild processing and to the extreme heat of the gun explosion process. The latter value is 81.4% of the former, equivalent to a heat injury to the contained protein of 18.6%.

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## WPB Restrictions on Laboratory Equipment

WPB reduced the number of types of laboratory equipment that may be sold or delivered only upon authorization by amendment to Order L-144, issued October 22.

Types of laboratory equipment which are in short supply are still subject to control: analytical balances (sensitivity 0.05 mg. or more sensitive); centrifuges valued at more than \$80 each; hydrogen-ion meters, electrometric type; metallosopes and metallographs; microscopes, stereoscopic wide field; Abbe refractometers; spectrograph (quartz), spectrophotometers (quartz), and spectrometers (infrared) and vacuum pumps (one micron or higher vacuum).



# Determination of Crude Lipid in Vegetable Matter

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method is proposed for extracting crude lipid from vegetable products. The complete procedure including preparation of the sample can be carried out in a relatively short time. The method yields considerably larger quantities of crude lipid from certain types of vegetable material such as immature seeds than do the commonly accepted procedures for crude fat. It is equally well adapted to wet and dry ground products.

**M**EASUREMENT of maturity in vegetables is of importance to the grower, processor, and consumer. Maturity at harvest may affect yield of raw product, processing behavior, and quality and yield of final product. Many vegetables store starch, which can be measured as an index of maturity. Soybeans, however, do not store starch, and sweet corn presents analytical difficulties due to its glycogen content (2). These and other vegetables increase in lipid content as they mature and a simple method for the determination of crude lipid might serve as an index of maturity.

In common methods for determining crude fat or ether extract, the sample is dried, ground, and extracted with petroleum ether for a number of hours. In some cases the extracted matter is then reground and the extraction repeated. The petroleum ether is then evaporated and the extract weighed. These procedures are time-consuming, especially in the case of material with high water content and products which have to be reground in a mortar with sand. The drying of the samples, particularly those which are immature and contain highly active enzyme systems, may introduce errors due to the actions of these enzymes at the elevated temperatures of drying.

A method by which an analysis, including the preparation of the sample, can be carried out in a relatively short time has been developed and is presented here. The method is simple and involves the use of standard laboratory equipment. The chief departures from common methods for crude fat determination are: the use of a Waring Blendor or comparable disintegrator for comminuting the sample, use of acetone as an initial extracting solvent, and elimination of extractors. The extract is taken up with petroleum ether in the latter part of the method; the material finally weighed is only that which is soluble in petroleum ether. The method has proved highly satisfactory on materials analyzed comparatively by both procedures, but its indiscriminate use, especially on hard oil seeds which may not be disintegrated by the proposed procedure, is not recommended.

## ANALYTICAL PROCEDURE

**REAGENTS.** Redistilled acetone. Redistilled petroleum ether, boiling range 35° to 59° C. (Skellysolve F). Sodium chloride. Filter aid.

**APPARATUS.** Aside from beakers the only apparatus required is a Waring Blendor or similar device, three 125-ml. suction flasks, two 60-ml. Büchner-type funnels with fritted-glass filter disks of medium porosity, and a source of vacuum.

One of the fritted-glass filters is used for the initial aqueous-acetone extraction and the other for clarifying petroleum ether solutions of lipids. Water and other substances contributing to the turbidity of petroleum ether solutions of lipids are likely to be drawn through a bare fritted-glass filter, especially if a high vacuum is employed. To ensure a clear filtrate, the fritted surface of the filter is coated with about 0.5 gram of Super Cel or other suitable diatomaceous earth, and a low vacuum is maintained in the system by the aid of a bubble bottle. The bubble bottle consists of a suction flask partly filled with water and having its side tube connected with both the vacuum line and the filtering system. A glass tube which passes through a one-hole stopper in the flask is adjusted so that its lower end is immersed to a depth of 5 or 7.5 cm. (2 or 3 inches) in the water. When the vacuum is turned on until bubbles of air are drawn through the water, a constant, reduced pressure exists in the system.

**PROCEDURE.** Grind 100 grams or more of the vegetable with an equal weight of water in a Waring Blendor for 3 to 5 minutes. If dehydrated products are to be used, soak 6 to 8 hours in 6 parts of water before disintegrating.

Weigh 5 grams of the resulting puree in a 10-ml. beaker and add 25 ml. of acetone from a pipet, stirring constantly. Transfer to a fritted-glass filter which has been connected by a cork stopper with a suction flask and apply suction until the liquid above the solids has been drawn into the flask, but interrupt the vacuum while the solids still appear distinctly wet.

Wash the beaker with about 5 ml. of acetone, scraping most of the solids from the sides into the liquid, and pour the material into the filter. Stir the contents of the filter with a glass rod having a rounded tip and apply suction as before. Wash the cake in the filter with 4 more 5-ml. portions of solvent, stirring after each addition and interrupting the vacuum before each new addition of acetone. Suction should be continued after the last 2 washings, however, until the cake in the filter appears dry.

Transfer the contents of the suction flask to a 250-ml. beaker and add about 1 gram of sodium chloride to prevent the formation of emulsion in the next step. Add a boiling stone, place the covered beaker on a steam bath, and evaporate most of the acetone. This will require about 15 minutes. Cool, and add 5 ml. of petroleum ether from a pipet, washing down the sides of the beaker. Rotate gently so as to expose all the lower surface of the beaker to the solvent, until masses of fat have disappeared. In some instances this may require several minutes. Carefully decant the petroleum ether layer into a fritted-glass filter coated with filter aid and apply mild vacuum. Repeat the petroleum ether extraction with two more 5-ml. portions.

The aqueous salt solution still retains a small amount of lipid in the form of an emulsion. To recover this, dissolve by adding 5 ml. of acetone and then replace the beaker on the steam bath to evaporate the fat solvent. Most of the formerly emulsified lipid will now be found floating on the water surface or adhering to the beaker at the water line. Cool and extract with three more 5-ml. portions of petroleum ether as before. Discard the salt solution and wash the lipid from the outside of the beaker lip into the filter with petroleum ether.

After the filter has drained, wash it with five 1-ml. portions of solvent, rotating the filter after each addition of petroleum ether to bring the diatomaceous earth into suspension. The rotating procedure prevents channeling of the solvent. In most instances, the diatomaceous cake will remain sufficiently porous to permit its use for several filtrations without renewal. Transfer the contents of the receiver to a 50-ml. tared beaker and evaporate the solvent by placing the beaker in an enameled pan on a steam bath until the odor of petroleum ether has disappeared. Cool the beaker and weigh. (If preferred, a tared 50-ml. flask can be used and the solvent removed by connecting with vacuum at room temperature until constant weight is obtained. Results will be substantially the same by both methods.)

Table I. Typical Crude Lipid Determinations on Undried, Immature Soybeans and Sweet Corn

Sample No.	Soybeans, Blanched	Soybeans, Unblanched	Sweet Corn, Blanched	Sweet Corn, Unblanched
Per Cent of Crude Lipid				
1	5.42	4.79	1.75	0.98
2	5.43	4.73	1.74	0.99
3	5.33	4.70	1.78	0.96
4	5.38	4.66	1.72	0.99

## RESULTS

As illustrated in Table I, results of crude lipid analyses in quadruplicate by the aqueous-acetone method are in good agreement. In the course of a considerable number of determinations on soybeans, sweet corn, and lima beans, results in duplicate seldom differed by more than 3% of the total crude lipid present. It has been found, however, that the percentage of lipid obtained by this method is consistently higher than that resulting from extraction with petroleum ether in the Soxhlet apparatus. The lipid ratio for the two methods is approximately constant for any one product, but may vary greatly between different products or



Table II. Extraction of Crude Lipids from Various Products and Modifications of the Same Product

Product	A Petroleum Ether Extraction by Soxhlet	B Extraction of Thimble Residue by Proposed Method	C Extraction by Proposed Method	$\frac{B}{A} \times 100$	$\frac{C - (A+B)}{C} \times 100$
	Per cent lipid <sup>a</sup>				
Soybeans, mature, blanched	18.56	2.14	21.23	10.1	+2.5
Soybeans, immature, blanched	14.96	3.03	18.15	16.8	+0.9
Soybeans, immature, unblanched	9.93	5.22	15.06	34.5	-0.6
Sweet corn, immature, blanched	4.94	1.08	6.00	18.0	-0.3
Sweet corn, immature, unblanched	3.23	1.25	4.60	27.9	+2.0
Copra meal	6.69	0.29	7.21	4.2	+2.0
Ripe olives, flesh, canned	70.77	0.37	70.35	0.5	-1.1
Peanuts, shelled, roasted	49.92	1.37	50.37	2.7	-1.8
Avocado, flesh, ripe	58.81	1.25	59.31	2.1	-1.3

<sup>a</sup> Dry basis.

between samples of the same product when one has been modified by a processing treatment, such as blanching. Results shown in Table II illustrate these variations.

Extraction of lipid in the Soxhlet apparatus was carried out as follows:

Approximately 10 grams of 40-mesh material previously dried to constant weight in a vacuum at 70° C. were folded in a large No. 2 Whatman filter. To facilitate drainage of the petroleum ether solvent and to increase the extraction rate, the Soxhlet thimble holding the wrapped sample was supported 1.25 cm. (0.5 inch) above the bottom of the thimble tube by a glass stopper (4). Rapid extraction was continued for 4 hours, at which time the sample was removed and thoroughly ground with an equal weight of sand in a large mortar. After it was replaced in the Soxhlet apparatus, the material was again extracted for a period of 4 hours. If turbidity was observed in the Soxhlet receiving flask, the solution was clarified by filtration with the fritted-glass filter as described above. Several samples were extracted for an additional 8 hours. Less than 0.5% of the total lipid extracted was obtained during this period.

In order to isolate the petroleum ether-soluble material obtained by the aqueous-acetone method but not extractable in the Soxhlet apparatus, the thimble residue after extraction was transferred to a fritted-glass filter and repeatedly treated with an equal weight of water, followed by extraction with acetone. After extraction the acetone was evaporated and the lipid taken up with petroleum ether in the manner described previously.

By reference to Table II it will be seen that the lipid value obtained by the Soxhlet extraction plus that obtained by the proposed method on the Soxhlet thimble residue equals approximately the value obtained on the original material by the proposed aqueous-acetone method.

On the basis of the percentage of lipid obtained from soybeans (Table II), it appears that the petroleum ether-soluble material remaining in the sample after Soxhlet extraction is greater in immature beans than in the mature product. In a similar way the proportion of lipid retained by the sample after Soxhlet extraction is greater in unblanched soybeans and unblanched sweet corn than in the comparable blanched vegetable.

In view of the higher results obtained with the aqueous-acetone method as compared with the Soxhlet apparatus, some of the tests commonly employed for the identification of a fat were applied to three types of petroleum ether extract obtained from blanched, immature soybeans and sweet corn. The results are shown in Table III. The methods used were official procedures of the Association of Official Agricultural Chemists (1) except that somewhat lower quantities of lipids were employed than those recommended.

It is clear from Table III that the material obtained from the Soxhlet thimble residue of the petroleum ether extraction is actually of a fatty nature and not merely an oil-soluble substance of unknown constitution, although some significant differences in values are observed. For example, their saponification num-

bers are above 200 for both soybeans and sweet corn, where the bulk of the total lipids is somewhat under this figure. Iodine values of the residual lipids, on the other hand, are low. The presence of low-molecular-weight saturated acids, such as butyric, lauric, and myristic, is suggested (3). Butyric acid is strongly indicated because of the high content of soluble acids in the saponified acids, and this is borne out by the low Hehner value. The somewhat high free fatty acid content of the Soxhlet residue is noteworthy.

To determine whether a combination of carbohydrates and proteins was responsible for any of the increase in yield, tests were applied to the petroleum ether-soluble material extractable in water and acetone after extraction by the usual procedure in the Soxhlet. The nitrogen content of material extracted from corn was 0.36% and from soybeans, 0.86%. The phosphorus content of the material extracted from corn was 1.1% and from soybeans, 2.5%. Sugar tests, including the phenylhydrazine test, Benedict's, and the orcinol test, all gave negative results. The reducing value with alkaline ferricyanide was small and showed only a slight increase after 90 minutes of hydrolysis with 1 N sulfuric acid. If it is assumed that the nitrogen found was present as lecithin, the corn extract would contain 20% and the soybeans 48% of this lipid. Since the molecular ratio of nitrogen to phosphorus was less than one, it is probable that protein was not present.

This investigation has not revealed why lipids are not entirely extracted from dried vegetable products by petroleum ether in the Soxhlet apparatus. One observation has been made, however, which may contribute to an explanation of this fact. When acetone is evaporated from water-acetone extracts in the aqueous acetone method a certain amount of a material having a waxy appearance separates out in the saline solution along with the lipid. This material appears to be insoluble in petroleum ether. It is also nearly insoluble in water, acetone, and 95% alcohol, but is readily soluble in either 50% aqueous acetone or 50% aqueous alcohol. Should some of the lipid particles in a dried vegetable be entirely enveloped in this substance it appears unlikely that petroleum ether could extract them. In the aqueous-acetone method, on the other hand, any lipid surrounded by this waxy material would be liberated by the solvent action of water-acetone mixtures employed for the initial extraction.

The relationship between the maturity and the percentage of

Table III. Results of Characterization Tests

	Soybeans			Sweet Corn		
	Petroleum ether Soxhlet	Proposed method, on thimble residue	Proposed method	Petroleum ether Soxhlet	Proposed method on thimble residue	Proposed method
Total lipid, %	14.96	3.03	18.15	4.94	1.08	6.00
Saponification No.	194	220	196	196	204	197
Iodine No.	139	88	132	105	74	98
Hehner No.	91.2	66.6	85.8	92.4	62.2	90.1
Mean molecular weight of insoluble nonvolatile acids	288	318	289	291	321	301
Soluble acids as % butyric	3.2	12.8	5.6	2.4	15.1	6.4
Free fatty acid as % oleic	1.5	6.7	2.7	1.4	8.5	2.7

Table IV. Relationship of Maturity of Soybeans and Sweet Corn to Lipid Content on Wet Basis

Soybeans		Sweet Corn	
Harvest date	% lipid	Harvest date	% lipid
9/16/42	3.76	8/18/42	0.98
9/19/42	4.25	8/21/42	1.28
9/22/42	4.56	8/24/42	1.39
9/28/42	4.81		
9/18/42	3.74	8/20/42	0.87
9/21/42	4.10	8/24/42	1.06
9/25/42	4.34	8/25/42	1.06
9/30/42	4.97		
		8/20/42	1.03
		8/24/42	1.28
		8/26/42	1.37



aid in soybeans and sweet corn has been mentioned previously. Typical data are shown in Table IV. The maturity range studied was such that prime quality, from the standpoint of edibility a green vegetable, fell at about the middle of the range.

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## Determination of Copper and Zinc in Their Naphthenates

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Two methods are described for the estimation of copper and zinc in the naphthenates of these metals. The first method is applicable to the analysis of either petroleum solvent solutions or aqueous emulsion of the naphthenates, while the second is limited to the analysis of emulsions. (1) The naphthenate is hydrolyzed by heating with hydrochloric acid, the liberated naphthenic acids are separated, and the metal content of the remaining aqueous portion determined by the usual volumetric methods. This procedure gives higher and more consistent results than are obtained by igniting the sample and determining the metal content of the ash. (2) The naphthenate emulsion is hydrolyzed by heating with hydrochloric acid, and the volume of the liberated naphthenic acids is measured. This volume is converted into terms of the metal content of the original emulsion by multiplication by a suitable factor. This method is recommended for routine control of processing baths.

THE copper and zinc salts of naphthenic acids have recently attained some prominence as rotproofing compounds for the treatment of fabrics and cordage. These compounds are usually applied as a solution in petroleum solvents or as an aqueous emulsion of such solutions.

A survey of the literature reveals no method for the determination of copper or zinc in such solutions or emulsions. Methods are available, however, for the determination of copper (2) and zinc (3) in fabrics proofed by these processes, and these methods have been applied to the analysis of solutions or emulsions of copper or zinc naphthenates: A measured sample is taken, the solvent burned or evaporated off, the residue ignited at a controlled temperature, the ash extracted with acid, and the metal content of the acid solution determined volumetrically.

A method similar to the above has been employed by Gottsch and Grodman (4) in the determination of cobalt, manganese, lead, and zinc in paint driers and boiled linseed oil. These authors have reported low and inaccurate results in the case of lead and zinc, and point out that at best the ashing method requires careful manipulation and strict control of the ignition temperature. They attribute their low results in the case of lead to fusion of the metallic oxide with the glaze of the crucible. It has been observed by the present authors that copper behaves in a similar manner if the ignition temperature is too high. In the case of zinc the low results are believed to be due to sublimation.

In addition to these considerations, the ashing method is subject to error due to loss of sample by spattering during the ignition. This is particularly true of the emulsions where the water must be evaporated off before igniting the residue.

Thus, while the ignition method will give accurate results with proper manipulation, the time and skill required to obtain such results render it somewhat unsatisfactory as a control method.

#### LABORATORY METHOD

A method has been developed in these laboratories which, in addition to being rapid, gives higher and more consistent results than are generally obtained by the ignition method re-

ferred to above. This method consists essentially of heating a measured sample of the solution or emulsion with hydrochloric acid until complete hydrolysis of the naphthenate is brought about. The naphthenic acids and solvent separate as a clear oily layer and the metal in the form of the chloride dissolves in the lower aqueous layer. The two layers are separated and the copper or zinc is determined by the usual volumetric method (5, 6).

**PROCEDURE.** A sample of the solution or emulsion estimated to contain 50 to 100 mg. of the metal is weighed (or pipetted) into a 250-ml. beaker and 10 ml. of 1 to 1 hydrochloric acid are added. The mixture is boiled for 2 to 3 minutes on a hot plate with rapid stirring. When the hydrolysis is complete the naphthenic acids dissolved in the solvent present separate as a clear, light yellow layer. (The completeness of hydrolysis under this treatment was proved in the following manner: The ether extracts from six determinations were combined, the ether was distilled off, and the residue was ignited. There was no visible ash, and no trace of either copper or zinc was detected.) The contents of the beaker are cooled, transferred to a separatory funnel, and 20 to 25 ml. of petroleum ether are added. The lower aqueous layer is drawn off into the original beaker, the ether layer is washed twice with 15-ml. portions of distilled water, and the washings are added to the beaker.

After the final washing and when the two layers have begun to separate out, a small quantity (3 to 5 ml.) of isopropyl alcohol is added. This helps to give a clear separation of the layers in cases where there is a tendency for emulsions to form at the interface.

The metal content of the aqueous acid solution is then determined by any of the standard volumetric methods.

**Determination of Copper.** The aqueous solution is made alkaline with ammonium hydroxide and a slight excess of glacial acetic acid is added, followed by about 2 grams of potassium iodide. The solution is titrated with sodium thiosulfate, starch indicator being added as the end point is approached. A convenient concentration of the sodium thiosulfate solution for use with the recommended sample weight is 12 grams per liter. This solution will have a copper factor of about 3 mg. of copper per ml., and should be standardized against pure copper.

This method has been found to give accurate results on solutions of pure copper naphthenate in petroleum solvent. After hydrolysis with hydrochloric acid, dilution of the organic layer with petroleum ether, and separation of the two layers, no trace of copper was found in the ether layer. It was therefore assumed that all the copper originally present was now contained in the aqueous layer. This solution was saturated with hydrogen sulfide, the precipitate was filtered off and redissolved in acid, and the copper content was estimated volumetrically. These results were in very close agreement with those obtained on the same solution using the hydrolysis method, excluding the hydrogen sulfide precipitation.

It is possible, however, that in actual practice there may be organic matter present which would tend to cause erroneously high results. Such organic matter may be added to the bath in the form of textile finishing agents, or may be picked up by the bath from fabric already processed. In cases where the presence of such organic matter is known or suspected, the procedure should be modified in the following manner:



After separation from the ether layer the acid solution is diluted to 200 to 300 ml. and saturated with hydrogen sulfide. The precipitated copper sulfide is filtered off on a sintered-glass crucible, washed with water, and redissolved in aqua regia. A few drops of sulfuric acid are added and the solution is evaporated to dryness. The residue is redissolved in 50 to 75 ml. of distilled water containing a few drops of sulfuric acid, the solution is made alkaline with ammonium hydroxide, and the procedure is continued as above.

**Determination of Zinc.** The aqueous solution is made alkaline with ammonium hydroxide, neutralized to phenolphthalein with 6 *N* hydrochloric acid, and 6 ml. more of the acid are added. Two drops of ferrous sulfate solution (2.5 grams per liter) are added, and the solution is diluted to about 200 ml. and heated to boiling. The solution is titrated while hot with potassium ferrocyanide solution, using the "split beaker" technique—i.e., a small portion of the solution is set aside and the remainder titrated rapidly. When the end point has been passed the small portion is returned to the beaker and the titration continued more cautiously until the exact end point is reached. As the titration proceeds the solution becomes a deep blue color which fades sharply to a pale green at the end point. A convenient concentration for the potassium ferrocyanide solution is 13 grams per liter, which yields a solution having a zinc factor of about 3 mg. of zinc per ml. The solution should be standardized against pure zinc.

Table I. Comparison of Analytical Methods

Sample	Metal Content	
	Ignition method	Hydrolysis method
	%	%
Copper naphthenate solution in petroleum solvent (solution A)	1.18	1.31
	1.27	1.31
	1.29	1.31
	1.25	1.31
	1.27	..
Copper naphthenate solution in petroleum solvent (solution B)	1.53	1.64
	1.51	1.64
	1.45	1.63
	1.46	..
Copper naphthenate, aqueous emulsion	3.27	4.18
	3.08	4.23
	3.55	4.24
	3.16	..
Zinc naphthenate solution in petroleum solvent	7.44	8.17
	7.26	8.17
	7.27	8.21
	7.38	8.23
	7.78	..
Zinc naphthenate, aqueous emulsion	4.53	4.70
	4.49	4.67

Table II. Determination of Conversion Factors

Sample	Metal Content by Hydrolysis Method		Volume by Acid Bottle Method	Factor
	Copper <sup>a</sup>	Zinc <sup>a</sup>		
	%	%	Units	
Copper naphthenate				
Emulsion A	0.446	...	44	0.010
Emulsion B	0.604	...	61	0.010
Emulsion C	0.654	...	65	0.010
Zinc naphthenate				
Emulsion D	...	0.130	10	0.013
Emulsion E	...	0.258	20	0.013
Emulsion F	...	0.234	25	0.013
Emulsion G	...	0.464	36	0.013

<sup>a</sup> Percentages expressed as grams of metal per 100 ml. of emulsion.

A comparison of the results obtained by the ignition method and the hydrolysis method on various solutions and emulsions of copper naphthenate and zinc naphthenate is given in Table I.

Examination of Table I shows that the hydrolysis method gives results which are higher and in general more consistent than those obtained by the ignition method. These discrepancies in the results obtained by the ignition method are believed to be due to mechanical losses during ashing of the sample. The hydrolysis method is rapid, requiring only about 30 minutes for a complete analysis, and involves no specialized equipment.

#### PROCESS CONTROL METHOD

A rapid method has been devised for the estimation of copper or zinc in aqueous emulsions of the naphthenates of these metals.

The method is recommended for use in process control, since minimum of equipment is required and a determination can be completed in 20 to 25 minutes. Such a method is of value in the treatment of fabrics by the emulsion process. Since there is gradual depletion of the metal content of the processing bath, it is necessary to make frequent analyses in order to maintain the metal concentration at the required level.

The determination is based on hydrolysis of the naphthenate measurement of the naphthenic acids produced, and estimation of the metal content by use of a suitable factor. The method outlined is applicable to the analysis of emulsions having a concentration range of approximately 0.01 to 1.00% of the metal. It may be applied to the analysis of more concentrated emulsions by suitable dilution of the emulsion before sampling.

A flask similar to that used for measuring the sulfuric acid absorption of petroleum products (1) is used for the determination. The flask has a capacity of approximately 50 ml. exclusive of the graduated neck, which has a capacity of 10 ml. and can be read to 0.1 ml.

Fifty milliliters of the emulsion are measured into the flask, 8 to 9 ml. of 1 to 1 hydrochloric acid are added, and the flask is thoroughly shaken. It is then placed in a bath of boiling water for 15 to 20 minutes, or until there is a clear separation of the two layers. The flask is cooled to room temperature and the volume of the upper layer is read. The percentage of metal represented by a unit volume of the upper layer is obtained by comparison with the metal content of the emulsion as determined by the hydrolysis method. This factor will be constant for all emulsions made by aqueous dilution of any one stock, but will vary slightly for concentrated emulsions from different sources. This is explained by the fact that petroleum solvent is used to dissolve the naphthenate before making up the concentrated emulsion; the volume of upper layer obtained in this analysis will thus depend on the amount of solvent used. The appropriate factor should therefore be calculated by determination of metal content of the same stock emulsion by the hydrolysis method.

For example, a sample on analysis by the hydrolysis method showed a zinc content of 0.464%. This same emulsion by the "acid bottle" method gave a reading of 36 units. The factor is therefore  $\frac{0.464 \times 1}{36} = 0.013\%$  zinc per unit. Each fresh batch

of stock emulsion must be checked in this manner and if an appreciable difference in the factor is obtained, the hydrolysis method rather than the acid bottle method should be applied to the analysis of dilute emulsions made from a mixture of two stock emulsions. To demonstrate the accuracy of the method, factors were calculated for various dilutions of concentrated emulsions (Table II).

The data presented in Table II indicate that an analysis by this method is accurate to two significant figures, which is sufficient to serve as a means of process control. In addition the procedure is rapid, requires a minimum of equipment, and may be performed by unskilled personnel.

#### ACKNOWLEDGMENTS

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# Photometric Determination of Silica in Aluminous Materials

## By the Molybdenum Blue Reaction

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A photometric method, based on the molybdenum blue reaction, is described for the determination of silica in sodium aluminate solutions and calcined alumina, and of silicon in metallic aluminum. Adjustment of pH is made with an indicator, thereby eliminating the necessity for a pH meter. The effect of aluminum salt concentration upon color development is minimized by the use of two calibration curves. The method may be used for the estimation of silica content from 0.01 to 1.0% in calcined alumina by varying the size of the sample. A precision of 4% at the optimum amount of silica determinable by this method may be expected when the method is applied to homogeneous samples.

THE rapid and accurate determination of silica in sodium aluminate solutions and calcined alumina, and of silicon in aluminum, is an important factor in the evaluation of alkaline processes for the production of aluminum. The usual gravimetric determinations not only are time-consuming but may yield low results because of the solubility of silica in the acid used for dehydration (2). Several procedures have been described for the use of the molybdisilicic acid reaction (1) in the determination of silicon content of aluminum. Pavelka and Morth (5) used the molybdenum blue reaction (3, 4, 6) for the microanalysis of aluminum for silicon and phosphorus. Possible interference of color-producing impurities in sodium aluminate solutions upon the determination of silica by the molybdisilicic acid method made it desirable to determine silica by the molybdenum blue method. This paper describes a rapid and accurate method based on this reaction.

### APPARATUS

A filter photometer (Fisher AC Electrophotometer) fitted with 650-m $\mu$  red filter and 2-cm. absorption cells was used for color measurements. A glass electrode apparatus (Leeds & Northrup Universal pH potentiometer) was used for the pH determinations.

### REAGENTS

**HYDROCHLORIC ACID** (1 + 9). Dilute 50 ml. of the concentrated acid to 500 ml.

**ACETIC ACID BUFFER**. Mix one volume of glacial acetic acid with two volumes of water.

**THYMOL BLUE INDICATOR SOLUTION**. Dissolve 0.4 gram of thymol blue in 10 ml. of freshly prepared 5% sodium hydroxide solution contained in a 300-ml. platinum dish. Dilute to 250 ml. and neutralize with dilute hydrochloric acid to an orange color; avoid an excess, which would precipitate the indicator. Transfer the solution to a 500-ml. flask and dilute to the mark.

**AMMONIUM MOLYBDATE SOLUTION**. Dissolve 25 grams of the salt,  $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$ , in 250 ml. of water. Allow to stand 4 hours and filter. Do not allow solution to stand over a week before using.

**SODIUM SULFITE SOLUTION**. Dissolve 170 grams of the anhydrous salt in about 900 ml. of water. Filter and dilute to 1000 ml.

**ALUMINUM CHLORIDE SOLUTION**. Dissolve in water either 2.3 grams of the anhydrous salt or 94.8 grams of the hexahydrate, acidify with a few drops of hydrochloric acid, filter, and dilute to 500 ml. (10 ml.  $\approx$  0.4 gram of alumina).

**STANDARD SILICA SOLUTION**. Weigh out an amount of sodium metasilicate,  $\text{Na}_2\text{SiO}_3 \cdot 5\text{H}_2\text{O}$ , the silica content of which has been carefully determined gravimetrically, which will be equivalent to

0.0500 gram of silica and dissolve in 500.0 ml. of water (1 ml.  $\approx$  0.1 mg. of silica). Prepare this solution on the same day it is to be used. When making the gravimetric analysis, use a sufficiently large sample so that the error due to solubility of silica in the dehydrating acid will be kept to a minimum.

**BORIC OXIDE**. Ignite boric acid in a platinum dish.

### PROCEDURE

The method has a working range of from 0.0 to 1.0 mg. of silica per 250 ml. in the presence of from 0.0 to 0.4 gram of alumina. If a sample containing 0.1 gram of alumina be considered the minimum practicable size, silica in quantities up to 1% may be determined.

Alkaline decomposition methods were chosen for attack of solid samples because these methods convert the silica to a soluble form suitable for determination without danger of volatilization or dehydration. Although different methods are used to convert the samples to sodium aluminate solutions, the final step is identical for all materials listed.

**PREPARATION OF SAMPLES FOR ANALYSIS.** *Sodium Aluminate Solutions.* Transfer a suitable aliquot containing not more than 0.4 gram of alumina to a 250-ml. beaker containing 100 ml. of water and dilute to 170 ml.

*Calcined Alumina.* Transfer a sample of from 0.1 to 0.4 gram of alumina (depending upon the silica content) to a 125-ml. platinum dish, cover with 4 grams of anhydrous sodium carbonate and 0.7 gram of boric oxide, and mix intimately by stirring with a platinum wire. Fuse at 1000° C. with a blast lamp, or preferably in a muffle furnace, until a perfectly clear melt is obtained. A 15-minute fusion time is usually sufficient for complete decomposition of the material. Cool, cover the melt with 50 ml. of water, and digest on a steam bath until the melt dissolves. Cool to room temperature and transfer the solution to a 250-ml. beaker containing 50 ml. of water. Rinse the dish, add the washings to the beaker, and dilute to 170 ml.

*Aluminum Metal.* Transfer a sample of from 0.05 to 0.20 gram (depending upon the silicon content) to a 125-ml. platinum dish, add 50 ml. of water and five pellets (about 0.6 gram) of sodium hydroxide. Let stand until the sample disintegrates, then add a few drops of 3% hydrogen peroxide, and digest for 15 minutes on a steam bath. Cool to room temperature and transfer the solution to a 250-ml. beaker containing 50 ml. of water. Rinse the dish, add the washings to the beaker, and dilute to 170 ml.

**DETERMINATION OF SILICA.** Immediately following the transfer of the alkaline solution to the beaker, add 8 drops of thymol blue indicator and add concentrated hydrochloric acid, dropwise, until the aluminum hydroxide, which first precipitates, is nearly dissolved and the solution has a yellow color. Do not bring the color of the indicator to red. Add 1 + 9 hydrochloric acid dropwise, stirring constantly, until the aluminum hydroxide completely dissolves and the indicator assumes a permanent pink color. This operation is critical in the adjustment of pH and may require as much as 5 minutes' time with samples of higher aluminum salt concentration. Add 5.0 ml. of 1 + 9 hydrochloric acid, 5.0 ml. of 1 + 2 acetic acid, and 5.0 ml. of ammonium molybdate solution in the succession named. Stir the solution between additions of reagents and stir vigorously for 1 minute after the addition of the molybdate reagent. Wait 5 minutes for the molybdisilicic acid to develop, then transfer to a 250-ml. volumetric flask. Reduce the molybdisilicic acid by adding slowly from a pipet, with vigorous shaking, 20 ml. of 17% sodium sulfite solution. Eight minutes after the addition of sulfite, add 5.0 ml. more of 1 + 2 acetic acid, dilute to the mark, and mix thoroughly. Thirty minutes after the addition of the sulfite, determine the color intensity of the solution with a photometer fitted with a red filter. Use distilled water in the reference cell. (When applying the method to highly colored liquors from the Bayer process, use an equivalent portion of this solution in the reference cell.) Read the amount of silica present from the proper cali-

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**Table I. Effect of Aluminum Salt Concentration upon pH Adjustment and Color Development<sup>a</sup>**

Al <sub>2</sub> O <sub>3</sub> Present, Gram	pH before Molybdate Addition	pH after Transmittance Measurement	Transmittance, %
0.00	1.38 ± 0.03	4.24 ± 0.00	61.5 ± 0.0
0.08	1.41 ± 0.04	4.18 ± 0.00	61.0 ± 0.1
0.20	1.38 ± 0.03	4.00 ± 0.01	61.9 ± 0.1
0.40	1.34 ± 0.05	3.72 ± 0.02	62.9 ± 0.1
0.60	1.26 ± 0.04	3.40 ± 0.02	61.6 ± 0.1
0.80	1.09 ± 0.01	3.12 ± 0.00	61.0 ± 0.1

<sup>a</sup> All values are averages for three determinations.

bration curve. Determine a blank on the reagents and subtract this value from the total silica to obtain the net silica in the sample.

**PREPARATION OF CALIBRATION CURVE.** To counteract the effect of aluminum salt concentration on color development, prepare two calibration curves based on 0.2 and 0.4 gram of alumina. Use suitable aliquots of the standard silica solution to determine 11 points on each curve (including the blank) in steps of 0.1 mg. of silica.

Dissolve four sodium hydroxide pellets (0.5 gram) in 50 ml. of water contained in a platinum dish and add the desired quantities of standard silica and aluminum chloride solutions. Transfer the alkaline solution to a 250-ml. beaker containing 50 ml. of water. Rinse the dish, add the washings to the beaker, and dilute to 170 ml. Continue the analysis as outlined under the determination of silica.

Construct a calibration curve, plotting the silica concentration against the color intensity. Draw a curve beginning at 100% transmittance, or zero extinction, parallel to the original curve which includes the silica derived from the sodium hydroxide and aluminum chloride, as well as the other reagents. This represents the true calibration curve and, of course, necessitates the determination and subtraction of a blank for each set of reagents used. The curves are slightly concave and their slopes vary with different amounts of alumina.

## EXPERIMENTAL

**ADJUSTMENT OF pH.** The determination of silica by the molybdenum blue reaction is dependent upon the amount of molybdisilic acid formed, which in turn is affected by variations in pH. It was established experimentally that for maximum development of molybdisilic acid the pH must be adjusted to between 1.0 and 1.4 before the addition of molybdate. Of the several techniques tried for the adjustment of pH the one described above proved most successful. Measurements of the pH of prepared solutions of varying alumina content used in the preparation of calibration curves and of actual samples indicated that the pH could be adjusted by the method described to  $1.35 \pm 0.1$  pH before the addition of molybdate. Typical data on pH obtained by this procedure are given in Table I.

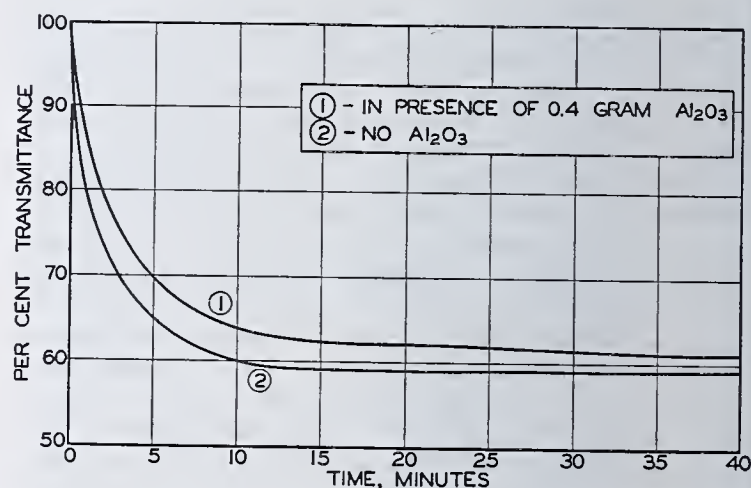
**PROGRESSION OF COLOR.** Kahler (4) pointed out that the use of acetic acid at a relatively high pH caused considerable color progression. This effect could not be disregarded if acetic acid were to be used to prevent the hydrolysis of aluminum salts. To check the color progression of molybdenum blue, with and without the presence of aluminum salts, solutions were prepared in the same manner as for the calibration curves. The molybdisilic acid formed by the addition of ammonium molybdate was reduced with sodium sulfite as described in the procedure, the solutions were immediately diluted to 250 ml., and transmittance measurements were made at several time intervals. Results of typical tests are shown in Figure 1.

It was found that the rate of color development levels off after 10 minutes, although the maximum intensity of color is not reached for several hours. The presence of aluminum salts also appeared to retard maximum color development. Although the results shown in Figure 1 were obtained on clear solutions, interference from hydrolysis of aluminum salts was encountered on some of the samples. This difficulty was met by varying the

procedure slightly to incorporate the addition of 5 ml. more of 1 + 2 acetic acid, 8 minutes after the addition of sulfite.

**EFFECT OF ALUMINUM SALTS.** Using the conditions outlined above, a series of tests was made to see what effect variations in aluminum salt concentration have upon color development. During these tests, 0.5 mg. of silica was added to each sample, and the addition of alumina was varied from 0.0 through 0.8 gram. These tests also included pH determinations before the addition of ammonium molybdate and after the measurement of transmittance. The results are given in Table I.

Apparently, increases in concentrations of aluminum salts, up to 0.4 gram of alumina, cause a corresponding retardation of color development. These variations, obviously too significant to be ignored, were minimized by the preparation of two calibration curves covering the range 0.1 to 0.4 gram of alumina. At least three factors may exert an influence upon the lower transmittance values found with the 0.6- and 0.8-gram alumina concentrations. These are lower initial pH values before addition of molybdate, lower pH during reduction with sulfite, and traces of silica from the aluminum salts. Difficulty in redissolving these larger amounts of alumina made continued study of these points inadvisable.

**Figure 1. Progression of Molybdenum Blue**

**EFFECT OF SODIUM AND BORON SALTS.** Before applying the method to the analysis of calcined alumina, the effects of larger amounts of sodium salts and of boron salts were investigated. Solutions similar to those used for the preparation of calibration curves and containing 2.6 grams of sodium hydroxide and 0.7 gram of boric oxide were analyzed for silica. The results, after subtraction of reagent blanks, indicated no effect from these fluxing reagents.

**EFFECT OF IRON SALTS.** Solutions identical with those used for checking the effects of boron and sodium, to which had been added 0.5 and 1.5 mg. of Fe<sub>2</sub>O<sub>3</sub> as ferric chloride, were analyzed for silica. No significant difference in results was observed.

**EFFECT OF PHOSPHORUS.** Solutions similar to those used for preparation of calibration curves and containing 0.2 gram of

**Table II. Effect of Phosphorus on Photometric Determination of Silica**

SiO <sub>2</sub> Added Mg.	P <sub>2</sub> O <sub>5</sub> Added Mg.	SiO <sub>2</sub> Found Mg.
0.20	0.2	0.21
0.20	0.5	0.22
0.20	1.0	0.24
0.60	0.2	0.61
0.60	0.5	0.65
0.60	1.0	0.70
1.00	0.2	1.02
1.00	0.5	1.07
1.00	1.0	1.11



alumina in each instance and varying quantities of silica and phosphorus pentoxide as phosphoric acid were analyzed in the usual manner. Results are shown in Table II.

The interference from phosphorus was considerable. However, the lowest amount tested was larger than the amount ordinarily encountered in 0.4 gram of alumina. Approximate corrections for phosphorus are probably feasible in the few instances where the phosphorus pentoxide content of the sample exceeds 0.2 mg.

#### APPLICATION TO SODIUM ALUMINATE SOLUTIONS

Two samples of highly colored sodium aluminate liquors from the Bayer process were analyzed gravimetrically by double dehydration with sulfuric acid and by the photometric method. Results are shown in Table III.

Table III. Comparative Analyses for Silica in Sodium Aluminate Solutions

Sample No.	SiO <sub>2</sub> , Grams per Liter	
	Gravimetric	Photometric
1 <sup>a</sup>	0.287 ± 0.005	0.287 ± 0.002
2 <sup>a</sup>	0.319 ± 0.014	0.326 ± 0.001

<sup>a</sup> Average of three values.

#### APPLICATION TO CALCINED ALUMINA

Three samples of calcined alumina previously analyzed by the standard gravimetric method, which involves decomposition of the sample with potassium pyrosulfate and double dehydration of the silica with sulfuric acid, were analyzed by the photometric method (Table IV).

#### APPLICATION TO ALUMINUM METAL

Three samples of aluminum metal were analyzed gravimetrically by double dehydration with sulfuric acid after decomposition with sodium hydroxide and hydrogen peroxide and by the photometric method. The results are shown in Table V.

For comparative purposes, the data in Tables III, IV, and V were reported to one significant figure more than is justified by the accuracy of the methods used.

#### PRECISION AND ACCURACY

The precision of the method is illustrated best by the analysis of the sodium aluminate solutions. On the basis of the results in Table III and similar results from the analysis of less highly colored liquors, the method is believed to have a precision within 0.02 mg. of silica. On the basis of the optimum amount of silica determinable, 0.5 mg., this represents a precision of 4%.

Table IV. Comparative Analyses for Silica in Calcined Alumina

Sample No.	SiO <sub>2</sub> , Per Cent	
	Gravimetric <sup>a</sup>	Photometric <sup>b</sup>
1	0.041 ± 0.001	0.041 ± 0.011
2	0.076 ± 0.010	0.077 ± 0.006
3 <sup>c</sup>	0.256 ± 0.008	0.294 ± 0.009

<sup>a</sup> Average of three values.

<sup>b</sup> Average of six values.

<sup>c</sup> Sample 3 contained 0.57% of P<sub>2</sub>O<sub>5</sub>. Difference in results is attributed to positive error caused by presence of P<sub>2</sub>O<sub>5</sub>.

This degree of precision was not attained in the analysis of alumina and aluminum. A comparison of the results in Tables IV and V, obtained by both gravimetric and photometric methods, indicates that the precision of the two methods is essentially the same. Possible heterogeneity of the samples may have affected results obtained when using both the gravimetric and photometric procedures.

The data in Tables IV and V indicate that, although there is good agreement between the two methods when applied to alumina, consistently high results are obtained when the photo-

metric method is applied to aluminum. This discrepancy cannot be explained by incomplete oxidation of silicon, since clear solutions resulted when the sodium hydroxide-hydrogen peroxide solutions of the samples were acidified with sulfuric acid.

An explanation of the discrepancy between the results obtained in the analysis of alumina and those obtained in the analysis of aluminum may be derived from consideration of (a) the loss of silica during a double dehydration with sulfuric acid, and (b) the silica content of the analytical reagents used. Hillebrand and Lundell (2) reported consistently low results in the gravimetric determination of silica, and attribute the losses to the solubility of silica in the dehydrating acid (hydrochloric or sulfuric). The error inherent in the gravimetric method was verified experimentally by analyzing solutions of aluminum sulfate to which known amounts of silica had been added. Low results were obtained in every analysis. The principal reagents entering into the analyses were those used to effect dissolution of the samples—namely, potassium pyrosulfate for the alumina and sodium hydroxide for the aluminum. Both reagents contained traces of silica detectable by spectrographic means. Since the quantity of reagent used with the alumina was about 12 times that used with the aluminum, the silica loss during dehydration of the alumina solution may have been compensated by reagent impurity, whereas no such compensation occurred in dehydration of the solution of aluminum. In view of this probability, together with the fact that the discrepancies between the gravimetric and photometric analyses of aluminum represent differences of the order of only 1 mg. of silica per 5 grams of alumina, it was concluded that the results obtained photometrically probably represent more nearly the absolute silica content of aluminous materials.

Table V. Comparative Analyses for Silicon in Aluminum

Sample No.	Si, Per Cent	
	Gravimetric	Photometric
1 <sup>a</sup>	0.094 ± 0.008	0.117 ± 0.001
2 <sup>a</sup>	0.259 ± 0.005	0.293 ± 0.013
3 <sup>b</sup>	0.278 ± 0.008	0.297 ± 0.004

<sup>a</sup> Average of six values.

<sup>b</sup> Average of three values.

#### DISCUSSION

The method described makes use of the molybdenum blue reaction for determining silica under carefully controlled conditions. Variables are compensated for by means of calibration curves for combinations of reagents that simulate actual samples. Some of the conditions were chosen arbitrarily; others were determined experimentally.

A number of calibration curves for solutions of varying concentrations of aluminum salts were prepared. Although the curves for 0.1 and 0.2 gram of alumina were sufficiently concordant to be considered identical, an appreciable difference was noted in the curves based on 0.08 and 0.40 gram of alumina. This made advisable the use of more than one calibration curve. When curves are constructed for 0.2 and 0.4 gram of alumina per 250 ml., samples containing from 0.0 to 0.4 gram of alumina may be analyzed with reasonable accuracy. For more precise work it is necessary to prepare calibration curves covering the range 0.0 to 0.1 gram of alumina per 250 ml.

No attempt has been made to apply this method to aluminum alloys.

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# VISCOSITY MEASUREMENT

## Master Viscometers

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This paper describes the construction and operation of two types of master viscometers designed for accurately calibrating the various types of routine viscometers now in extensive use. The first instrument is calibrated directly with water at 20° C. The remaining instruments of larger bore are then calibrated against the first by means of hydrocarbons of suitable viscosity. These calibrated master viscometers are used to establish accurately the viscosity of a whole series of calibrating fluids to be used in calibrating routine viscometers. Since some fluids change in viscosity with age, the master instruments are employed to check the standard fluids two or three times per year. The opaque-type master is useful in handling very dark or opaque liquids and in studying the drainage effect in viscosity measurements.

IN TWO previous papers (3, 4) simple and accurate routine viscometers were described for the measurement of the viscosities of liquids ranging from one third to more than two thousand times the viscosity of water. A special design (4) is necessary for the case where the test liquid is so dark or opaque that the operator cannot see through glass that is wet with a thin film of it.

Before these routine viscometers are usable it is necessary to calibrate them accurately. This paper describes suitable master viscometers which were designed and are now used by many laboratories for this special purpose. Obviously, it is not necessary for each laboratory to secure master viscometers, since calibrated routine viscometers are available through scientific supply companies. However, many large laboratories desire to maintain complete calibration facilities and for them master viscometers are recommended. It is unnecessary to use master viscometers in the calibration of the special-type routine viscometer for nonviscous liquids (viscosity range of 0.3 to 2 centipoises) shown as Figure 2 in reference (3), since these can be calibrated directly with water (1).

### OPERATION OF MASTER VISCOMETERS

These instruments are used in exactly the same manner as the routine viscometers (3, 4); in fact, the only major difference is in capillary length and the height of driving liquid head. Most of the errors encountered in viscometry are caused by a loss of driving head and can be reduced in magnitude by having a large driving head. In this respect the master viscometers have four times the head available in the routine type. When made with the dimensions shown they will fit readily into a constant-temperature bath 60 cm. (24 inches) deep.

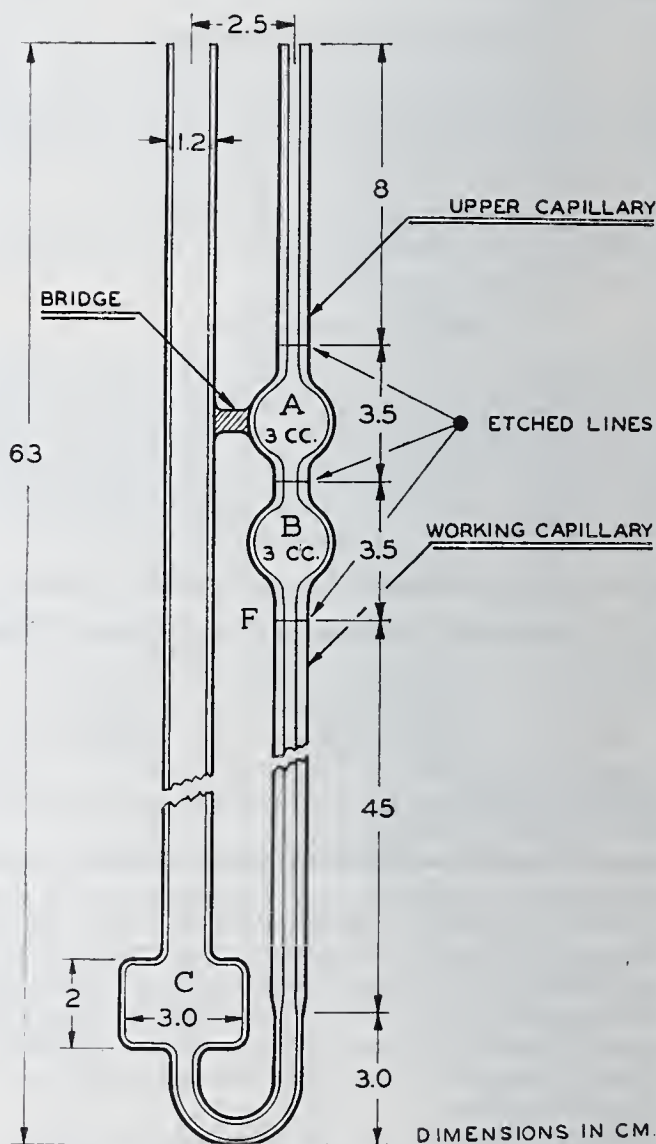
In order to charge, the instrument is held in an inverted vertical position with the capillary side submerged in the liquid under test. Suction is then applied to the other arm of the instrument and the meniscus is brought to mark  $F$ . The instrument is then revolved to its normal vertical position and placed in the constant-temperature bath. It may be quickly aligned to a vertical position with the aid of a small plumb bob made from a piece of silk thread and a small piece of lead wire attached to a small cork to fit into the 1.2-cm. viscometer leg.

When the bath temperature is attained suction is applied to raise the meniscus into bulb *A*, Figure 1. The efflux time is then measured for the liquid to discharge from between the etched marks above and below bulb *B*. Check runs are made by repeating this procedure. When using the master instrument (Figure 2) it is necessary to close the upper capillary with a short piece of rubber tubing and pinchclamp after allowing the fluid to drain from the upper capillary into *A*; times are measured for filling bulbs *C* and *D* after bath temperature is attained. Viscosities calculated by the constants for *C* and *D* should agree.

Unlike the master viscometer of Figure 1, check runs can be made with the opaque type only by cleaning and reloading. The opaque type has the advantage of no drainage errors and opaque liquids can be tested. It has the disadvantages of longer time to attain bath temperature (as much as five times as long as the other type), since the oil is stagnant during heating, and a lengthy time for check runs. In general, the normal type shown as Figure 1 is preferable, but if one wishes to study drainage, handle opaque liquids, or increase rates of shear by applied external pressure the opaque type is best. Where external pressures are applied to increase shearing rate, drainage errors would be serious in the normal-type viscometer.

### CALIBRATION PROCEDURE

The viscosity of water is probably known to a higher degree of accuracy than any other liquid. Its complete stability and general availability make it an excellent reference liquid for all relative viscosity measurements. Therefore, a master viscometer of small capillary bore is calibrated by means of distilled water at 20° C. where the kinematic viscosity of water is 1.007 centi-



**Figure 1. Master Viscometer, Normal Type**



stokes. This figure may be inaccurate to the extent of  $\pm 0.5\%$ . However, if all laboratories use this figure relative viscosities can be measured with a higher degree of accuracy, since the measurement of relative viscosities is much simpler than the measurement of absolute viscosities. Fortunately, practically all viscometers in use in this country and abroad have been calibrated using this primary reference viscosity of 1.007 cs. at 20° C., thanks largely to the efforts of the viscosity subcommittee of the American Society of Testing Materials. In addition, values of 0.689 centistoke for water at 100° F. (37.78° C.) and 0.518 centistoke at 130° F. (54.44° C.) are recommended by A.S.T.M.

When the small-bore master viscometer has been calibrated with water, a more viscous hydrocarbon may be tested in it and this in turn used to calibrate a second master viscometer of larger capillary bore, which could not be calibrated directly with water because of high capillary velocity with resulting low efflux time and a high and inaccurately known kinetic energy correction. A third and fourth master viscometer of successive larger capillary bores may then be calibrated in a similar manner. In order to avoid cumulative error in this step-up procedure, each may be further checked against the first master viscometer by means of suitable oils. When the fourth is checked against the first it means that the efflux time may be 4 minutes in the fourth and 24 hours in the first. While this procedure is lengthy, it need be done but once each year when master viscometers are checked.

When the master viscometers are calibrated, a series of oils or pure hydrocarbons is then tested and their viscosities are accurately established. These standardized fluids are then employed for the daily, weekly, or monthly calibrations of routine viscometers such as the Cannon-Fenske type (3, 4), the Ubbelohde type (8), the Zeitfuchs type (9), or any of the other accurate routine viscometers now in extensive use.

The calibrating fluids should be carefully selected or prepared. Many lubricating oils increase in viscosity from 0.5 to 1.0% per year by aging at room temperature. In general, these can be stabilized by desludging with aluminum chloride or by solvent refining followed by the addition of a suitable oxidation inhibitor. Since the oils are stored at room temperature stabilizing is not difficult. A series of high viscosity index stabilized oils in use since 1933 has not changed in viscosity by 0.2% to date. It is preferable to store in glass rather than metal cans and it should be a laboratory rule that when a sample of oil is removed from storage it should be discarded after use.

The number of oils to be standardized for use as calibrating fluids will vary with the needs of laboratories. The writer finds a series of 14 such oils ranging in viscosity from 1 to 3000 centistokes by a rough factor of 2 between each adjacent pair (1 cs., 2 cs., 4 cs., etc.) to be very convenient. One can operate with fewer oils but calibrations will require a longer time.

It is necessary to calibrate a viscometer at only one temperature, since the change of viscometer constant with temperature is very small and can be calculated (3). This variation of constant with temperature amounts to only 0.5% for a change of 43.33° C. (110° F.) in the routine type (3, 4) as proved by experiment. It is much smaller in the master type described here. This change in constant with temperature is due to a change in volume of liquid in the instrument with temperature change since the viscometers are charged at room temperature.

#### MAGNITUDE AND SOURCE OF ERRORS

Viscosity is usually calculated from efflux times by means of Poiseuille's equation corrected for kinetic energy loss as follows:

$$KV = \frac{\omega}{\rho} = \frac{\pi g H r^4 t}{8 L V} - \frac{m V}{8 \pi L t}$$

This is usually given as:

$$KV = Ct - \frac{B}{t}$$

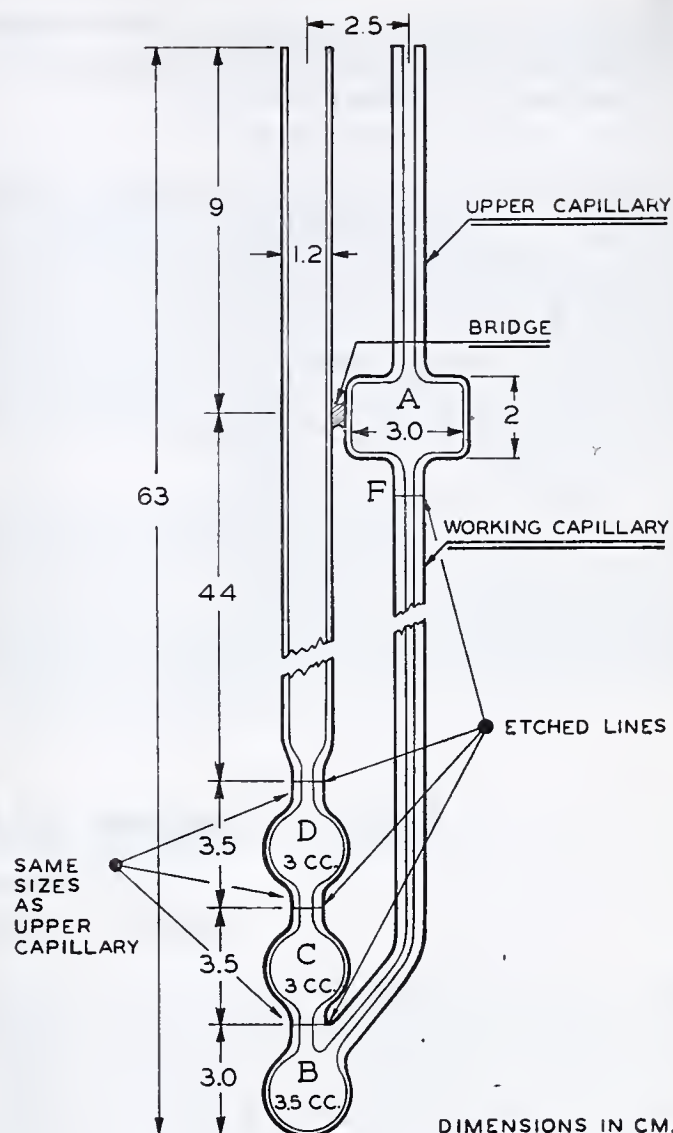


Figure 2. Master Viscometer, Opaque Type

where  $KV$  = kinematic viscosity in stokes

$\omega$  = viscosity in poises

$\rho$  = density in grams per cc.

$g$  = gravitational constant in cm. per second per second

$H$  = driving fluid head in cm.

$r$  = capillary radius in cm.

$t$  = efflux time in seconds

$L$  = capillary length in cm.

$V$  = efflux volume in cc.

$m$  = kinetic energy correction coefficient

$$C = \frac{\pi g H r^4}{8 L V}$$

$$B = \frac{m V}{8 \pi L}$$

Hundreds of experiments have shown that  $C$  is a constant for long capillary viscometers. However,  $B$  will be a constant only if  $m$ , the kinetic energy correction coefficient, is a constant. From a theoretical standpoint one would not expect  $m$  to be a constant and recent extensive measurements indicate that  $m$  varies with Reynolds number as well as with the shape of the capillary entrance and exit. If one employs a symmetrical viscometer the value of  $m$  will be different for flow to left than for flow to right. Bingham and Geddes (2) and Spooner and Serex (7) have made such measurements and found wide variations in  $m$ —for example, (2) an  $m$  of 1.46 for flow to left and an  $m$  of 0.74 for flow to right, and (7) an  $m$  of 3.05 for flow to left and an  $m$  of 1.52 for flow to right. This indicates the extreme sensitiveness of this loss of driving force at the capillary ends. It is, of course, not surprising to find a different value of  $m$  for different flow directions, since the exit end of the capillary contributes more to  $m$  than does the entrance end. Consequently, if there are slight differences in the shape of the two ends  $m$  will vary with direction of flow.

For the master viscometers described here and the routine viscometers previously described, where all capillary ends are



gradually tapered, experiments show that  $m$  does not exceed 0.5. However, since it does vary, the safest procedure is so to design the viscometers that the term  $B/t$  is negligible compared to the term  $Ct$  and the above equation then becomes

$$KV = Ct$$

If fluids of widely different viscosities leave different quantities of liquid on the walls of the efflux bulb for routine viscometers (3) and the master viscometer shown as Figure 1, then  $C$  will not be a constant, since this drainage will change  $V$ . The master viscometer shown as Figure 2 is free of drainage error, since the liquid is entering clean dry bulbs. On all oils investigated in the two master-type instruments it was found that  $C$  is a true constant. This means that the rate of drainage is inversely proportional to the viscosity. Thus, a 300-centistoke oil will drain at only one third the rate of a 100-centistoke oil but the total drainage time will be three times as great for the 300-centistoke oil and so in each case the degree of drainage is the same.

The equations and methods for calculating the various corrections in basic calibration work have been presented in detail (3), and are not repeated here. The magnitude of the corrections for the master viscometer shown as Figure 1 is: (a) kinetic energy correction 0.04% for water at 20° C., less for more viscous fluids; (b) surface tension correction when using water and hydrocarbons in the same instrument, 0.09%; (c) change of viscometer con-

stant with temperature, 0.03% per 30° C. change in temperature. For the master viscometer shown as Figure 2 the same figures apply, except (c) is 0.1% per 30° C. since the charge is greater.

Results are reproducible in these instruments to within 0.1% and consequently relative viscosities can be measured to that degree of precision. Absolute viscosities depend upon the error inherent in the value of 1.007 centistokes taken for water at 20° C. This is probably in the order of  $\pm 0.5\%$ . These master viscometers are an improvement over those described earlier (5, 6). The capillary diameter required for a given constant  $C$  can be calculated from the equations above.

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## Gum Content of Distillate Diesel Fuels

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THE gum content of distillate Diesel fuels is of less significance in their evaluation than is this property of gasolines; there are, however, occasions when it is necessary to compare two Diesel fuels with respect to their tendency to form gum. Furthermore, the advent of increasing quantities of cracked Diesel fuels, complicated by the catalytic action of various metals with which such fuels may come in contact, makes it pertinent to establish a method by which the gum content, preformed and potential, may be determined.

The following conditions for the evaporation of the fuel were established as satisfactory, after testing out numerous variations.

#### APPARATUS

Erlenmeyer flask, capacity 50 ml. Condenser, Liebig type, water-cooled, with adapter, preferably sealed on. Filtering flask, capacity 1 liter. Oil bath. Source of inert gas (natural, artificial, nitrogen, carbon dioxide). Vacuum pump.

#### PROCEDURE

Transfer 25 ml. of the Diesel fuel to a tared 50-ml. Erlenmeyer flask. Connect the flask to the condenser and to the source of inert gas by means of a cork stopper fitted with two glass tubes, the ends of which are flush with the bottom of the cork. Attach the filtering flask to the condenser adapter by means of a rubber stopper, and connect the side arm of the flask to a source of vacuum. Apply a vacuum of 50 to 55 cm. (20 to 22 inches) of mercury to the assembly, and pass gas into the system at a rate of approximately 250 ml. per minute.

Immerse the flask in an oil bath heated to that temperature at which the sample will be evaporated practically to dryness in 45  $\pm$  5 minutes. (This temperature will generally be approximately 150° F. below the 90% point of the A.S.T.M. D158 distillation.) At the end of the specified time increase the bath temperature 50° F., and increase the rate of gas flow to approximately 500 ml. per minute. Maintain these conditions for 10 to 15 minutes; then remove the oil bath, shut off the vacuum, and increase the flow of gas until the pressure in the system is approximately atmospheric.

Disconnect the tared Erlenmeyer flask, add 25 ml. of a mixture of equal parts of carbon tetrachloride and acetone to the flask, and evaporate to dryness on the steam bath. Connect the flask to the condenser as before, again apply the vacuum, and with the gas flow and temperature as previously established for

the end phase of the evaporation heat the flask for approximately 15 minutes. Disconnect the flask as previously described, clean the outside thoroughly, and weigh.

$$\text{Mg of gum per 100 ml.} = \text{mg. gain in weight} \times 4$$

Following the procedure outlined samples of Diesel fuels and various distillate fractions of similar boiling range were tested, primarily to establish the repeatability of the method over as wide a range as was possible with the stocks available. The data are presented in Table I.

#### VARIABLES INVESTIGATED

The procedure described constitutes a purely empirical test, as are all similar methods for measuring gum in petroleum products. It is therefore necessary that all details of the test be standardized and followed if results of useful precision are to be obtained.

The following variables were investigated in arriving at the selected test conditions.

**DEGREE OF VACUUM.** A vacuum of 50 to 55 cm. (20 to 22 inches) of mercury was found adequate to volatilize 25 ml. of fuel within an hour, varying the temperature to suit the stock and keeping this to a reasonable maximum. With this degree of vacuum there is little danger of collapsing the flask, and it is easier to avoid trouble from leaks.

**EVAPORATION TIME.** Polymerization under the conditions existing in the test is a function of both time and temperature. The total period during which the sample is heated is set at about one hour. This period was arbitrarily selected, and further experience with the test, using a wider range of fuels, may show that the heating period can be reduced or that a longer period would be more satisfactory.

**AMOUNT OF SAMPLE.** The amount of sample selected, 25 ml., is sufficient to give a weighable residue with the Diesel fuels available to the authors, and it behaved satisfactorily in the apparatus selected. The residue from smaller samples must be multiplied by a larger factor, and small errors, obviously, would be proportionally magnified.



Table I. Gum in Petroleum Products

	Cracked Naphtha Rerun Still Bottoms		Gas Oil			Com- mer- cial Diesel Base, Un- treated	Gas Oil		Commercial Diesel, Treated			Gas Oil (Cracked)
	Untreated	Treated	A	B	C		D	E	A	B	C	
Gravity, ° A.P.I.	29.2	28.9	33.1	31.4	31.8	38.3	34.3	29.1	38.8	39.1	41.2	28.4
Distillation, A.S.T.M. D158												
10%	442	442	560	430	436	378	483	614	390	398	390	456
50%	480	480	596	522	530	436	538	650	432	438	427	503
90%	573	573	653	640	648	510	579	660	504	510	492	611
End point	617	617	693	715	719	564	616	692	560	565	548	654
Gum, mg. per 100 ml.	82 86	27 26	30 29	70 70	60 58	8 6	18 22	40 41	12 14	8 9	8 9	52 48
Evaporation tempera- ture, ° F.												
Start	400	400	500	450	450	350	400	500	350	350	350	450
Final	450	450	550	500	500	400	450	550	400	400	400	500

Table II. Results of Tests

Sample	Residue Mg./100 ml.
Fuel Sample 1	
Without oxidation	8 12 Av. 9 8
5 hours in bomb, no catalyst	16 16 Av. 19 20
5 hours in bomb, iron and brass catalyst	88 92 Av. 90 90
Fuel Sample 2	
Without oxidation	124 128 Av. 125 124
5 hours in bomb, no catalyst	212 216 Av. 215 216
5 hours in bomb, iron and brass catalyst	1464 1452 Av. 1458 1460

**RATE OF GAS FLOW.** In order to keep oxidation and polymerization at a minimum the oil vapors should be removed speedily from the flask. A current of inert gas does this satisfactorily. Natural gas was used in this work because it was available and cheap, but nitrogen or carbon dioxide would be equally satisfactory. Artificial gas varies considerably in composition and purity; however, there seems no reason why it would not serve the purpose as well as natural gas.

The rate of gas flow does not appear to be critical. At 250 ml. per minute the velocity is just sufficient to sweep out the oil vapors. A higher rate of gas flow accelerates the evaporation, but oil was carried over mechanically and there was some cooling of the flask, both factors causing erratic results. However, it was found that the gas flow could be increased to advantage when the evaporation was practically complete.

**TEMPERATURE OF EVAPORATION.** It is obvious from the distillation range of oils covered by the general term "distillate Diesel fuels" that it is not possible or at least not desirable to specify a single bath temperature for the test, if a limiting evaporation period is also specified. From the standpoint of polymerization a uniform temperature for all fuels is desirable; however, this would force completion of the evaporation of certain samples at an undesirably rapid rate, whereas the evaporation of other fuels would be unduly prolonged. The authors' work indicated that the temperature of the 90% point of the A.S.T.M. D158 distillation minus 150° F. correlated fairly well with the desired evaporation period, at least sufficiently to give a good lead to the operator testing an unknown stock.

**RE-EVAPORATION OF RESIDUE.** The residue obtained by simply evaporating to an apparently oil-free condition contained varying amounts of oil held by a surface layer of nonvolatile gum.

Solution of the residue in acetone-carbon tetrachloride, and re-evaporation to dryness, eliminated this source of error and yielded repeatable results. On the oils used a single such treatment was sufficient; however, it may be desirable to repeat this, giving a check on the approach to "constant" weight.

**NONVOLATILE RESIDUE OTHER THAN GUM.** Diesel fuels may contain small amounts of lubricating oil or other nonvolatile oil-soluble substances, either added intentionally or present as the result of contamination. The residue obtained by the procedure described will obviously include such material; hence, the entire "gum" content may not be deleterious. No satisfactory method of distinguishing between gum—i.e., the

product of the oxidation and/or polymerization of hydrocarbons normally present in the Diesel fuel boiling range—and other nonvolatile material has been found.

#### ACCELERATED TEST

Having established a reasonably repeatable method for determining the "gum" in a sample by evaporation, various means of establishing an accelerated test, by which the rate of gum formation in stored Diesel fuel might be predicted, became available. The dominant factors operating upon an unstable fuel are heat, pressure, oxygen, and contact with catalytically active metals, all of which speed up the reactions which form gum. The possibilities in apparatus and range of conditions are endless, and the method which correlates most closely with service and storage conditions cannot be established until considerably more is known on the subject. For the authors' purpose it seemed suitable to subject the fuel to the same conditions as are set up by the Government for aviation gasoline—i.e., a bomb under 45 kg. (100 pounds) oxygen pressure, at 212° F., using a catalyst. Iron and brass were the catalysts selected, and in the tests reported below approximately 100 sq. cm. (15 square inches) surface, of both metals in each test bottle, were used; a 5-hour test was judged to be suitable; and a 200-ml. sample in a 235-ml. (8-ounce) sample bottle was taken. Obviously, in a standardized test the composition of the metal rods, as well as their surface area, should be specified.

Table II shows the results of tests made under these conditions, employing the previously developed evaporation method for measuring the gum formed.

Continuous pressure charts were made during the heating of these samples. Sample 1 showed practically no pressure drop; sample 2 without catalyst showed an early pressure drop, but the curve did not continue downward appreciably; the same sample, with catalyst, showed a similar early drop, which continued throughout the 5 hours. The indications of the pressure-drop curves are in line with the gum tests of the samples.

#### SUMMARY

The evaporation procedure selected is sharply repeatable even with such a high "gum" residue as 1400 mg. per 100 ml.—i.e., using successive portions of the same oxidized sample—and the authors believe that it gives a useful measure of nonvolatile gummy material dissolved in the oil when tested. They have no data as to the amount which can be tolerated in actual service.

The Army induction bomb and test with iron and brass catalyst may be used to obtain an accelerated test. The authors have no data on the correlation of this accelerated test with service.



# Determination of Lithium in Its Minerals

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Since the methods of J. L. Smith and Berzelius for the decomposition of minerals of the silicate type and the isolation of the alkali chlorides are unsatisfactory for the determination of lithium in its minerals, a combination of the two procedures is proposed. The sample is treated with hydrofluoric acid and most interfering elements are eliminated by precipitation with calcium hydroxide. The residue, which invariably occludes some lithium, is submitted to a fusion similar to that of Smith. Reprecipitation of all precipitates obtained in the course of analysis is essential. For the separation of lithium from the other members of the alkali family, gravimetric methods are unreliable and extraction methods require repeated treatments of the insoluble residue. Of the precipitation methods, the *n*-hexanol and 2-ethylhexanol methods are considered best for moderate amounts of lithium, retreatment of the insoluble precipitate being required for larger quantities of lithium. The *n*-butyl alcohol-hydrogen chloride method of Willard and Smith can be extended to the separation of lithium from both sodium and potassium. The proposed procedure is held superior to any other method used at the present time for the separation of lithium from sodium and potassium.

LIKE so many other metallic elements, lithium metal was scarcely known to the public at the beginning of this century, only 25 years ago making its entry into the field of industrial application. Large tonnages of the metal, its minerals, and chemical compounds, however, are produced today and are adsorbed notably by the glass, ceramic, air-conditioning, and metallurgical industries, but also in the electrical and pharmaceutical fields (25). Lithium has now been listed as essential by both the American and British war agencies (16).

The United States is the leading producer and consumer of lithium metal and apparently has the largest known ore reserves.

## COMPOSITION OF LITHIUM MINERALS

The composition of the principal lithium minerals is of importance to the analyst, in determining the proper analytical procedure to be followed.

### Main Lithium Minerals:

Spodumene,  $\text{LiAl}(\text{SiO}_3)_2$ , containing 4 to 8%  $\text{Li}_2\text{O}$  and generally a little sodium.

Amblygonite,  $\text{Li}(\text{AlF})\text{PO}_4$ , containing 8 to 9%  $\text{Li}_2\text{O}$ ; lithium may be partly replaced by sodium and fluorine by hydroxyl.

Lepidolite, composition variable, ranging from that of polythionite to that of lithium-bearing muscovite, approximating  $\text{KLi}[\text{Al}(\text{OH},\text{F})_2]\text{Al}(\text{SiO}_3)_3$  containing from 2 to 4%  $\text{Li}_2\text{O}$ .

Triphylite,  $\text{Li}(\text{Fe},\text{Mn})\text{PO}_4$ , containing from 2 to 4%  $\text{Li}_2\text{O}$ .

Petalite,  $\text{LiAl}(\text{Si}_2\text{O}_5)_2$ , containing from 2 to 4%  $\text{Li}_2\text{O}$ .

Zinnwaldite,  $\text{LiF}$ ,  $\text{KF}$ ,  $\text{FeO}$ ,  $\text{Al}_2\text{O}_3$ ,  $3\text{SiO}_2$ , containing 2 to 3%  $\text{Li}_2\text{O}$ .

### Somewhat Less Important Lithium Minerals:

Taeniolite,  $\text{KLiMg}_2\text{Si}_4\text{O}_{10}\text{F}_2$ .

Polythionite,  $\text{KLi}_2\text{AlSi}_4\text{O}_{10}\text{F}_2$ .

More than 100 other minerals contain small amounts of lithium.

## DETECTION OF LITHIUM

Volatile lithium compounds color the flame a bright crimson; the flame test may therefore be used to detect lithium in minerals. The interference of sodium can be overcome by the use of a proper color screen. In the presence of strontium, a little barium chloride added to the solution and examined in the flame will first show the crimson of lithium, then the green of barium, and finally, the red of strontium.

Spectroscopic methods have been proposed since 1912 for the detection and determination of minute quantities of lithium (13, 35), they are worthless, however, for quantitative analysis if weighable amounts of lithium are present.

In the flame spectrum, which is less sensitive to foreign elements than either the arc or spark spectrum, there are two sharply defined lines: a weak line  $\text{Li}\beta$  of wave length 6104 and a bright red line  $\text{Li}\alpha$  of wave length 6708. The presence of nearly  $10^{-6}$  mg. of lithium can be detected in this way. In the arc spectrum a blue line appears, besides eighteen other feebler lines (23).

## COMBINED J. LAWRENCE SMITH-BERZELIUS PROCEDURE FOR ISOLATION OF LITHIUM AND OTHER ALKALI METALS

In separating lithium from other elements with which it is associated in its minerals, too little attention is sometimes paid to the vast differences in the solubility of many lithium compounds compared with other alkali compounds. Lithium fluoride, oxalate, carbonate, and phosphate are so much more insoluble in water and in an excess of the respective reagents than are the corresponding sodium and potassium compounds, that numerous qualitative and quantitative methods based on the formation of lithium fluoride (2, 11), lithium carbonate (1, 10, 24), and lithium phosphate (3, 6, 22) have been proposed.

Unless special precautions are employed, considerable quantities of lithium may be lost if the scheme of operation recommended for the isolation of the other alkali metals is indiscriminately employed in the determination of lithium.

Despite this unique chemical behavior, otherwise excellent textbooks on mineral analysis (15, 31, 33) have failed to present an adequate and specific procedure for the initial decomposition of lithium minerals, but usually refer to two classical methods which are most valuable for the isolation of sodium and potassium—the J. Lawrence Smith method (37) or the even more ancient method of Berzelius (5).

Lundell and Hoffman (21) have noted that some lithium remains in the extracted residue from the J. Lawrence Smith fusion, and numerous tests carried out by this writer would indicate that igniting this extracted residue, transferring it to the platinum or nickel crucible originally used, and reheating it with more ammonium chloride—a cumbersome and very tedious procedure—will not always guarantee complete recovery of all lithium.

The Berzelius method, on the other hand, involving the use of hydrofluoric acid and sulfuric acid, and modifications proposed by Low (20), Krishnaya (19), and Scholes (32), are even less attractive. Appreciable lithium frequently remains in the extracted residue, partly due to occlusion, partly because of incomplete decomposition of the mineral. In addition, magnesium, if present, will accompany the alkalis, and its troublesome removal is always imperative, lest it be found with the final lithium sulfate, provided organic solvents are used for separating lithium from the other alkali metals.

The removal of large amounts of sulfate ion by precipitation as barium sulfate, which is apt to occlude considerable lithium (12, 39) is another drawback of the Berzelius method.

Of the alternative ways of eliminating the hydrofluoric acid, evaporation with perchloric acid or volatilization as hydrofluosilicic acid have been proposed (40). While these modifications, particularly the latter, are of considerable importance for the determination of sodium and potassium in feldspars or other silicate minerals known to be low in magnesium, they leave complete decomposition of lithium minerals as uncertain as before. Moreover, magnesium, if present, would accompany the lithium as in the original Berzelius method. As many lithium minerals contain large amounts of phosphate ion (amblygonite, triphylite) the removal of which is imperative, the above modifications of the Berzelius method cannot directly be applied to the determination of lithium.

Removal of fluoride ion by precipitation as calcium fluoride was suggested by Koenig (18). The method was severely criticized by Willard and co-authors (40), who tried it in the deter-



nination of sodium plus potassium in a high-potassium feldspar, claiming that the calcium hydroxide precipitate retained as much as 50 mg. of the 0.2440 gram of sodium plus potassium expected.

This writer carried out a number of experiments to test Koenig's method. While apparently no procedure involving the use of hydrofluoric acid achieves complete decomposition of certain lithium minerals, and Willard's criticism regarding retention of alkali by the calcium hydroxide precipitate seems justified, parts of Koenig's method are excellent and have found their way into the procedure developed by this writer.

In other methods used in the past Berzelius (4) decomposed lithium minerals of the silica type (spodumene, lepidolite, petalite, zinnwaldite) by fusion with twice their weight of calcium and barium carbonate. Troost (38) fused lepidolite with an equal weight of barium carbonate, half its weight of barium sulfate, and one third its weight of potassium sulfate. Weinland and Storz treated triphylite with aqua regia and Mueller (24) decomposed the same mineral with hydrochloric acid.

**NEW PROCEDURE.** The proposed procedure, combining the best features of the Berzelius method as modified by Koenig (18) with some parts of the J. Lawrence Smith method (37), starts by treating the sample with hydrofluoric acid, followed by the Koenig modification to eliminate the fluoride ion. The bulk of the lithium will be found in the water extract. The extracted residue, which is practically free from silica, contains comparatively small but frequently weighable amounts of lithium, owing to incomplete decomposition of the sample and occlusion by the calcium hydroxide, partly caused by incomplete conversion of the lithium fluoride into the hydroxide. The residue is ignited in a platinum crucible of the usual shape and is then submitted to a fusion similar to that proposed by J. Lawrence Smith. This fusion can be achieved, as silica has been largely removed by the initial hydrofluoric acid treatment, at a comparatively low temperature, say 700° C. The extracted residue from this fusion is always free from weighable amounts of lithium.

The method is applicable to all lithium minerals, of both silicate and phosphate type.

(Throughout all operations Pyrex beakers were used with good success, although some chemists claim that the best of glassware is attacked at slightly elevated temperatures, causing low lithium results when ammonium chloride salts are expelled in beakers.)

The amount of lithium which may be expected to be retained in the first calcium hydroxide precipitate, on account of incomplete decomposition of the sample and occlusion, is illustrated in Table I.

Table I. Retention of Lithium by Calcium Hydroxide Precipitate

Type of Sample	Lithium Oxide Found		
	In Ca(OH) <sub>2</sub> extract Mg.	In Ca(OH) <sub>2</sub> ppt. by occlusion Mg.	In Ca(OH) <sub>2</sub> ppt. (undecomposed sample) Mg.
Spodumene	25.3	2.7	4.3
	26.4	2.0	3.9
	18.9	3.2	4.4
Amblygonite	31.0	4.9	1.8
	32.3	4.6	2.4
	28.4	5.6	2.9

Transfer 0.5 gram of the 200-mesh sample, dried at 110° C., to 50- to 100-ml. platinum dish, moisten with water, add 25 ml. of hydrofluoric acid, and evaporate to dryness on the water bath. Add 10 ml. more of the hydrofluoric acid and repeat the evaporation, finally drying the salts at about 150° C. on a hot plate or in an electric oven.

Digest the residue with 25 ml. of hot water for about 5 minutes. Complete solution is rare when dealing with high-grade lithium minerals, then wash the solution quantitatively into a 250-ml. beaker containing 2 grams of calcium oxide in 75 ml. of water. Police and rinse the platinum dish, adding the washings to the beaker. (The calcium oxide used is prepared by ignition of the special grade of carbonate mostly used for the J. Lawrence Smith method.)

Boil contents of beaker for about 2 minutes, allow precipitate to settle for a short time, then decant the supernatant liquid through a 12-cm. No. 40 Whatman filter paper. Finally trans-

fer the precipitate onto the paper and wash it six times with hot water or, if the presence of magnesium is known or suspected, with hot water containing calcium hydroxide. Police the beaker, or remove any precipitate adhering to the beaker with a piece of filter paper moistened with dilute hydrochloric acid. Hold the residue (residue I).

To the filtrate, which has been received in a 400-ml. beaker, add 1 ml. of ammonia and two 1.25-cm. (0.5-inch) cubes of ammonium carbonate. Heat to boiling, filter immediately through an 11-cm. No. 40 Whatman filter paper, and wash the residue (residue II) with 1 to 50 ammonia containing some ammonium carbonate. Wipe the beaker with a small piece of filter paper moistened with dilute hydrochloric acid. Hold the filtrate (filtrate A).

Combine the papers containing residues I and II and ignite in a 30-ml. platinum crucible. Mix the residue intimately with 1 gram of ammonium chloride and cover the crucible with a tightly fitting platinum cover. Insert the crucible for two-thirds of its depth in a hole in an asbestos pad and heat it, at a low heat first, until the charge has melted or sintered.

Transfer the cold platinum crucible to a 250-ml. beaker, cover the crucible with hot water, and allow to stand in a warm place for at least 6 hours. Remove crucible from beaker and loosen and return to the beaker, with the help of a stirring rod or a policeman, any cake or precipitate adhering to the crucible. Heat solution to boiling, filter the supernatant liquid through a 12-cm. No. 40 Whatman filter paper into a 400-ml. beaker, finally transfer the residue onto the filter paper, and wash it, as specified above, with hot water or hot water containing calcium hydroxide. Discard the residue, which is free from lithium compounds.

To the filtrate add 1 ml. of ammonia and two 1.25-cm. (0.5-inch) cubes of ammonium carbonate. Heat to boiling, filter through an 11-cm. No. 40 Whatman filter paper, and wash the precipitate with dilute ammonia containing ammonium carbonate. Wash precipitate back into original beaker and dissolve it in a few drops of hydrochloric acid. Render the solution just ammoniacal and repeat the ammonium carbonate separation, finally filtering through original paper into the main filtrate (filtrate B). Discard the residue.

Combine filtrates A and B and evaporate to dryness on a water bath. When bone dry, volatilize the ammonium salts by placing the uncovered beaker on a hot plate and gradually heating to the full heat of the plate. Allow to cool, add 30 ml. of hot water to effect solution of the salts, then 4 drops of ammonia and 6 to 8 drops of saturated ammonium oxalate solution. Heat to boiling, allow precipitate to settle for one hour, then filter off on a 9-cm. No. 40 Whatman paper and wash six times with a 1% solution of ammonium oxalate.

Wash precipitate back into original beaker and dissolve it in a little hydrochloric acid. Repeat ammonium oxalate separation, finally filtering through original paper into main solution.

Evaporate solution, which has been received in a 250-ml. beaker to dryness on the water bath and volatilize the ammonium chloride as before.

(As lithium invariably must be separated at a later stage from the other members of the alkali family, the sulfate ion should be removed at this point because of the sparing solubility of lithium sulfate in most reagents employed. In case the barium sulfate precipitate appears small, it need not be filtered but should be removed with the excess of the barium in the subsequent ammonium carbonate separation.)

Take dry salts up with about 30 ml. of hot water and 3 drops of hydrochloric acid and, after heating to boiling, add dropwise sufficient 10% barium chloride to guarantee complete precipitation of the sulfate ion. Allow precipitate to settle for 2 hours, filter through a small No. 40 Whatman filter paper, and wash it ten times with hot water.

To the filtrate, in a 250-ml. beaker, add sufficient ammonia to render the solution ammoniacal, then two 1.25-cm. (0.5-inch) cubes of ammonium carbonate. Heat to boiling and filter off the barium carbonate, washing the precipitate with dilute ammonia-ammonium carbonate wash solution. Dissolve the precipitate in a few drops of hydrochloric acid and reprecipitate with ammonia and ammonium carbonate, finally filtering through original paper into main filtrate and discarding the precipitate.

Evaporate the combined filtrates, in a 250-ml. beaker, to dryness and volatilize the ammonium chloride salts on a hot plate. Take salts up with 40 ml. of water and small amounts of ammonia, ammonium carbonate, and ammonium oxalate. After heating the solution to boiling and allowing it to stand for at least 2 hours, filter the small residue on a small filter paper and wash it with dilute ammonia-ammonium carbonate solution. Wash the precipitate back into original beaker, dissolve in hydrochloric acid, and reprecipitate with ammonia, ammonium carbonate, and ammonium oxalate. Discard the final precipitate.

Evaporate the combined filtrates to dryness and volatilize



the ammonium chloride. Moisten the salts with dilute hydrochloric acid, again evaporate to dryness, and heat strongly on hot plate to expel any remaining ammonium chloride.

The dry salts obtained in this way consist of the combined chlorides of the alkali metals, possibly contaminated by a little ammonium chloride. If required, the ammonium chloride can be quantitatively removed by washing the above hydrochloric acid solution into a platinum dish, evaporating to dryness, and heating at a dull red heat over a Bunsen burner, but observing all precautions necessitated by the volatility of the alkali chlorides.

**CORROBORATION AND VERIFICATION.** In numerous lithium determinations of various lithium minerals, all residues or precipitates, marked in the above procedure as "discarded", were tested and found free from weighable amounts of lithium. In some cases, however, the residues, when examined by the flame test, gave a faint crimson color.

As no standard samples of lithium minerals have been available to this writer, artificial mixtures of lithium and the elements with which it is associated in minerals were made up. Lithium, after being isolated by the procedure described above, was finally weighed as the sulfate. In order to avoid the separation of lithium from other alkali metals—a subject discussed below—no potassium or sodium salts were introduced into the artificial mineral. A solution of lithium chloride was used in these experiments which was checked by pipetting out aliquot portions and determining their lithium content by evaporating with sulfuric acid and weighing as the sulfate (Table II).

Table II. Accuracy of Proposed Procedure

LiCl Taken Equivalent to $\text{Li}_2\text{SO}_4$	Artificial Mineral, in Addition to LiCl, Contained	$\text{Li}_2\text{SO}_4$ Found
Gram	Gram	Gram
0.0735	$\text{SiO}_2 = 0.1500$	0.0727
0.0735	$\text{Al}_2\text{O}_3 = 0.1500$	0.0731
0.0735	$\text{AlPO}_4 = 0.1000$	0.0740
0.1470	$\text{MgCl} = 0.0100$	0.1477
0.1470	$\text{Fe}_2\text{O}_3 = 0.0200$	0.1481
0.1470	$\text{CaF}_2 = 0.0200$	0.1466
0.2205		0.2193
0.2205		0.2200
0.2205		0.2204

#### REVISED AND EXTENDED *n*-BUTYL ALCOHOL-HYDROGEN CHLORIDE METHOD FOR SEPARATION OF LITHIUM FROM SODIUM AND POTASSIUM

In the usual course of analysis for the determination of sodium and potassium, lithium, if ignored, will cause high results for either sodium or potassium, depending on the procedure employed. If potassium is determined as the perchlorate or the chloroplatinate and sodium calculated from the combined chlorides of the alkali metals, lithium will count as sodium because of the solubility of its perchlorate or chloroplatinate in the reagents used. On the other hand, if sodium is determined as the triple acetate, part of the lithium will accompany the sodium while the remainder counts as potassium.

**PRECIPITATION OF LITHIUM.** Before the appearance of Gooch's article in 1887 (14) the most favored method was that of Berzelius (6) as modified by Mayer (22), which is based on precipitation of lithium as the phosphate. Today, the method is considered tedious in operation and incompatible with accuracy.

Carnot (11) took advantage of the sparing solubility of lithium fluoride in alcohol and dilute ammonia, compared with that of sodium fluoride. The method has some merits for the preliminary separation of lithium from sodium, prior to the precipitation of the latter as triple acetate (2) and for the preparation of pure lithium salts (29). Mueller (24) and more recently Caley (10) precipitated lithium as carbonate. Of the more recent methods in which lithium is precipitated, separation as potassium ferric periodate (27), as stearate (8), and as complex periodate (30) should be mentioned.

Some of the above methods are qualitative or semiquantitative, others are confined to the determination of small amounts of lithium, but none is suited for the quantitative separation and determination of lithium as it is found in most lithium minerals.

**EXTRACTION OF LITHIUM AND PRECIPITATION OF CHLORIDES OF OTHER ALKALI METALS.** Most methods in use today take advantage of the ready solubility of lithium chloride and the insolubility of the chlorides of the other members of the alkali family in various solvents or mixtures of organic solvents. The methods are of two types, distinguished by extraction of lithium chloride or precipitation of the other chlorides.

The first group comprises the larger number of methods:

The alcohol-ether extraction method of Rammelsberg (28).

The pyridine method of Kahlenberg and Krauskop (17).

The isobutyl alcohol method of Winkler (42).

The acetone method of Brown and Reedy (7).

The dioxane method of Sinka (34).

The main objection to methods in this category are their pronounced tendency toward occlusion of lithium chloride inside the crystals of the other alkali chlorides and also the formation of insoluble lithium hydroxide, thus necessitating repeated treatments of the insoluble residue. The methods are tedious in operation and error-inviting because of the number of manipulations involved.

The other general type of procedure consists in dissolving the mixed chlorides in a small amount of water from which the alkali chlorides, other than lithium chloride, are precipitated either by adding an organic agent, as in Palkin's alcohol-ether precipitation method (26), or by dehydrating the aqueous solution of the mixed chlorides with organic solvents of a high boiling point, as in the amyl alcohol method of Gooch (14) and the *n*-hexanol and 2-ethylhexanol methods of Caley and Axilrod (9).

In the hands of this writer, Palkin's method has not yielded satisfactory results, as many as three separations being required to separate 40 mg. of lithium oxide from 15 mg. of sodium oxide. The isoamyl alcohol method of Gooch "has the disadvantage that neither potassium nor sodium chlorides are quantitatively insoluble in isoamyl alcohol, so that relatively large corrections must be applied for the amounts of salts that dissolve along with the lithium chloride" (9). Caley and Axilrod's methods are excellent for the separation of small amounts of lithium chloride ( $\text{LiCl} < 60$  mg.) from moderate amounts of sodium and potassium chlorides. Because of the comparatively sparing solubility of lithium chloride in *n*-hexanol and 2-ethylhexanol (see Table III) the separation appears unsatisfactory, judging from the data given by the authors, for amounts of lithium chloride as low as 120 mg., unless two separations are carried out.

**THE *n*-BUTYL ALCOHOL-HYDROGEN CHLORIDE METHOD OF WILLARD AND SMITH.** The method of Willard and Smith (41) for the separation and determination of lithium and sodium, involving the use of *n*-butyl alcohol as a solvent for the lithium and a 20% solution of hydrogen chloride in *n*-butyl alcohol as a precipitant for the sodium, is a combination of the two types of methods cited above, in that sodium and lithium are first extracted from the mixed alkali perchlorates, sodium being precipitated in the extract.

The method has, despite its numerous excellent features, not found the recognition it deserves. This is largely due to the authors' insistence on confining their procedure to the separation and determination of lithium and failing to extend their investigation to the separation of lithium from potassium. It is therefore hardly surprising that Willard and Smith's method is not used in routine or control work for the determination of lithium in its minerals, and that its application has been largely confined to the quantitative separation and determination of lithium, sodium, and potassium. A condensed description of the latter process, as given by Smith and Ross (36), is here quoted to facilitate discussion of the method.

The combined perchlorates of potassium, sodium, and lithium, free from an excess of perchloric acid, are treated with a mixture of equal parts of *n*-butyl alcohol and ethyl acetate, two extractions with intermediate solution of the potassium perchlorate being required. Potassium is finally weighed as the perchlorate.



Table III. Solubilities of Chlorides and Perchlorates

Solvent	NaCl	KCl	LiCl
Grams per 100 grams of solvent			
Ethyl alcohol	0.0649	0.0294	2.54
Amyl alcohol	0.002	0.0007	9.03
<i>n</i> -Butyl alcohol	0.005	0.0030	12.98
<i>n</i> -Butyl alcohol containing 6% HCl + 0.5% HClO <sub>4</sub>	0.0007	0.0005	10.61
Isobutyl alcohol	0.00047	0.0008	10.57
<i>n</i> -hexyl alcohol	0.0010	0.00005	7.2
2-Ethylhexanol	0.0001	0.0000	3.7
Acetone	0.000	0.000	3.86
Pyridine	0.000	0.000	13.39
Dioxane	0.000	0.000	...
	NaClO <sub>4</sub>	KClO <sub>4</sub>	LiClO <sub>4</sub>
Grams per 100 grams of saturated solution			
Ethyl alcohol	12.82	0.012	60.28
<i>n</i> -Butyl alcohol	1.83	0.0045	44.23
Ethyl acetate	8.80	0.0015	48.75
50% <i>n</i> -butyl alcohol + 50% ethyl acetate	11.99	0.0025	...
Isobutyl alcohol	0.78	0.005	36.73

Table IV. Separation and Determination of Sodium and Lithium

NaCl Taken Grams	NaCl Found Grams	LiCl Taken Equivalent to Li <sub>2</sub> SO <sub>4</sub> Gram	Li <sub>2</sub> SO <sub>4</sub> Found Gram	Volume of Filtrate and Washings Ml.
0.0500	0.0498	0.0735	0.0738	40
0.1000	0.0997	0.0735	0.0737	40
0.1000	0.1003	0.7350	0.7354	40
0.2500	0.2492	0.2205	0.2211	40
0.4000	0.3994	0.2205	0.2209	50
0.6000	0.5995	0.2205	0.2216	60
1.0000	0.9990	0.2205	0.2212	75
1.2000	1.1991	0.2205	0.2213	85
1.5000	1.4989	0.2205	0.2215	100
2.0000	1.9989	0.2205	0.2213	100

Ethyl acetate must be expelled by evaporation before precipitation of sodium as sodium chloride from the *n*-butyl alcohol solution of the perchlorates of sodium and lithium is attempted, using for the latter separation a 20% solution of hydrogen chloride in *n*-butyl alcohol. The sodium chloride is either weighed as such or dissolved in water and its chlorine content determined volumetrically by Mohr's method.

Lithium is determined by evaporation of the *n*-butyl alcohol extract, and conversion of the lithium perchlorate into the sulfate, in which form it is weighed.

This writer has extensively used Willard and Smith's method for the separation of lithium from sodium, obtaining excellent results. It should be noted, however, that the authors, in their endeavor to determine both lithium and sodium quantitatively, specify the use of sufficient *n*-butyl alcohol to guarantee a clear solution (free from insoluble sodium perchlorate) before precipitation of sodium as sodium chloride.

Because of the sparing solubility of sodium perchlorate in *n*-butyl alcohol (see Table III) the authors recommend a minimum of 18.5 ml. of *n*-butyl alcohol for every 100 mg. of sodium chloride present. It is obvious that, when separating small amounts of lithium from 1 gram or more of sodium chloride, it is necessary to use inconveniently large quantities of both the *n*-butyl alcohol and the solution of hydrogen chloride in *n*-butyl alcohol. This, in turn, necessitates considerable corrections for the solubility of sodium chloride in the reagents employed.

Experiments carried out by this writer, recorded in Table IV, conclusively show that lithium can be quantitatively separated from larger amounts of sodium by precipitating the latter from considerably smaller volumes of *n*-butyl alcohol than those recommended by Willard and Smith. Prior to the precipitation, the possible presence of insoluble sodium perchlorate, caused by the sparing solubility of the latter in *n*-butyl alcohol, was disregarded. It was found that this insoluble sodium perchlorate, when boiled for one minute with the solution of hydrogen chloride in *n*-butyl alcohol, was quantitatively converted into sodium chloride which is practically insoluble in the reagents employed, as has been shown by Willard and Smith. In the experiments recorded in Table IV the reagents and manipulations suggested by Willard and Smith were used.

SEPARATION OF LITHIUM FROM POTASSIUM. Willard and Smith's method asks for the removal of potassium as perchlorate prior to the separation of lithium from sodium. As this involves considerable manipulations, an attempt was made to determine whether lithium could be separated from potassium and sodium in a single operation, using the 20% solution of hydrogen chloride in *n*-butyl alcohol as a precipitant.

Potassium salts, other than sulfates or phosphates, when fumed with perchloric acid, are converted into potassium perchlorate only slightly soluble in *n*-butyl alcohol. The effect of the hydrogen chloride solution in butyl alcohol upon the potassium perchlorate was studied by this writer, who found that potassium perchlorate is partly converted into potassium chloride which, however, is only very slightly soluble in butyl alcohol and even less in butyl alcohol containing hydrogen chloride.

To determine the extent of the conversion of potassium perchlorate into potassium chloride, varying amounts of potassium chloride were fumed to dryness with an excess of perchloric acid and the potassium perchlorate was dissolved in water and again evaporated to dryness in order to remove any perchloric acid which had been retained during the crystallization process. The dry salts were boiled, in one series of experiments for 1 minute and in a second series for 5 minutes, with a 6% solution of hydrogen chloride in *n*-butyl alcohol, then filtered on a Gooch crucible and washed with the same alcoholic solution of hydrogen chloride. The crucible was dried for a few minutes at 110°C. and finally for 15 minutes in a muffle at 350°C.

The crucible was weighed, the residue dissolved in hot water, the crucible dried and weighed again, and the chloride ion determined in the water extract volumetrically by Mohr's method. The calculated results are presented in Table V.

It is significant that the extent of the conversion of potassium perchlorate into potassium chloride seems to be independent of the amount of potassium perchlorate originally present, but depends mainly on the concentration of hydrogen chloride in the *n*-butyl alcohol. This indicates that the conversion is, as could be reasonably expected, caused by the slight solubility of potassium perchlorate in *n*-butyl alcohol: The soluble potassium perchlorate is precipitated as potassium chloride, causing the formation of more soluble perchlorate and further precipitation of the chloride, until, after prolonged boiling with the 6% solution of hydrogen chloride in *n*-butyl alcohol, the conversion presumably is complete. As the present investigation is primarily concerned with the determination of lithium, no attempt was made at this time to establish whether potassium perchlorate could be converted quantitatively into potassium chloride with a minimum amount of reagents and manipulations.

Table V. Conversion of Potassium Perchlorate into Potassium Chloride

KCl Taken Grams	KCl Found Gram	KClO <sub>4</sub> Found Equivalent to KCl Grams	Length of Boiling Min.	Volume of Filtrate Ml.
0.0500	0.0324	0.0174	1	35
0.0500	0.0389	0.0114	5	35
0.1000	0.0344	0.0659	1	40
0.1000	0.0435	0.0571	5	60
0.3000	0.0400	0.2608	1	60
0.5000	0.0422	0.4585	1	80
0.5000	0.0537	0.4470	5	80
1.0000	0.0418	0.9582	1	80
2.0000	0.0423	1.9580	1	85

The solubility of potassium chloride in anhydrous *n*-butyl alcohol containing 6% hydrogen chloride was found to be about 0.8 mg. per 100 ml. of solution (see Table VI). Hence, the solubility of potassium chloride is reduced from 2.5 mg. per 100 ml. of butyl alcohol to 0.8 mg. by the addition of 6% hydrogen chloride and to about 0.5 mg. by the further addition of 0.2 ml. of perchloric acid. Because of the limited amount of work done, the solubility data recorded here may be somewhat liberally interpreted.



**Table VI. Solubility of Potassium Chloride in Anhydrous *n*-Butyl Alcohol Containing 6% of Hydrogen Chloride**

(Note. Weighed quantities of potassium chloride were treated with a 6% solution of hydrogen chloride in *n*-butyl alcohol at a boiling heat. After cooling to 25° ± 1° C., the solution was filtered through a tared Gooch crucible, the residue transferred onto the Gooch and washed with the 6% solution of hydrogen chloride in *n*-butyl alcohol. The crucible was dried and weighed and any loss of weight noted.)

Volume of Filtrate and Washings Ml.	KCl Taken Gram	KCl Left Undissolved Gram	Loss of Weight Gram	Free HClO <sub>4</sub> Present %	Solubility of KCl in 100 Ml. Gram
35	0.2500	0.2497	0.0003	...	0.0008
74	0.4000	0.3993	0.0007	...	0.0009
98	0.5000	0.4996	0.0004	...	0.0008
104	1.0000	0.9993	0.0007	...	0.0007
87	0.5000	0.4996	0.0004	0.5	0.0005
126	0.5000	0.4994	0.0006	0.5	0.0005

In order to determine the total solubility of potassium perchlorate and potassium chloride in *n*-butyl alcohol containing 6% hydrogen chloride and to prove the accuracy of the proposed procedure, varying amounts of potassium chloride and lithium chloride were fumed with perchloric acid, the excess of the latter was expelled by evaporation, and the dry perchlorates were heated to boiling with *n*-butyl alcohol. Enough 20% solution of hydrogen chloride in *n*-butyl alcohol was added to bring the concentration of the hydrogen chloride to 6%. The cold solution was filtered through a tared Gooch crucible and washed with the 6% solution of hydrogen chloride in *n*-butyl alcohol. Lithium was determined in the filtrate by evaporating the butyl alcohol solution, finally weighing it as the sulfate.

The residue on the Gooch crucible was dissolved in hot water, the filtrate collected in a 250-ml. beaker was fumed to dryness with an excess of perchloric acid, and potassium was finally determined as the perchlorate, using the original Gooch crucible and applying reagents and manipulations recommended by Smith and Ross but making only one extraction with the mixture of ethyl acetate and *n*-butyl alcohol (see Table VII).

**Table VII. Separation and Determination of Potassium and Lithium**

KCl Taken Gram	KClO <sub>4</sub> Found Equivalent to KCl Grams	LiCl Taken Equivalent to Li <sub>2</sub> SO <sub>4</sub> Gram	Li <sub>2</sub> SO <sub>4</sub> Found Gram
0.0500	0.0498	0.0735	0.0737
0.0500	0.0502	0.2205	0.2200
0.1000	0.0995	0.2205	0.2211
0.2500	0.2503	0.2205	0.2202
0.5000	0.4994	0.2205	0.2214
1.0000	0.9999	0.2205	0.2205
1.0000	1.0004	0.4410	0.4403

The results in Table VII show that the separation of lithium from potassium by the *n*-butyl alcohol-hydrogen chloride method is very satisfactory, making it possible to extend the procedure to the separation of lithium from both sodium and potassium (see Table VIII). Because of the considerable solubility of lithium perchlorate and lithium chloride in *n*-butyl alcohol compared to the sparing solubility of lithium chloride in various other organic solvents recommended for this separation, and the very small solubility of sodium and potassium chlorides in *n*-butyl alcohol containing 6% hydrogen chloride, Willard and Smith's method, as revised and extended by this writer, should be considered superior to any other method used at the present time for the separation of lithium from sodium and potassium.

**SEPARATION OF LITHIUM FROM CESIUM AND RUBIDIUM.** Cesium and rubidium are rarely encountered in lithium minerals, with the exception of lepidolite, which sometimes contains the two alkali metals in such small quantities that no special provisions for their presence need be considered.

Preliminary work carried out by this writer would indicate that rubidium and cesium perchlorates are also affected by the solution of hydrogen chloride in *n*-butyl alcohol. Because of the smaller solubility of the perchlorates of rubidium and cesium in *n*-butyl alcohol, the conversion into the respective chlorides is smaller. The separation of lithium from rubidium appears to be good, while the separation from cesium seems less satisfactory.

**Procedure for Separation of Lithium from Sodium and Potassium.** The reagents used were identical with those recommended by Willard and Smith, with the exception of the *n*-butyl alcohol. This is now readily obtainable on the market of sufficient purity to do without the elaborate and time-consuming purification procedure employed by Willard and Smith.

The mixed chlorides of potassium, sodium, and lithium, obtained as described above, are dissolved in a little water, 5 ml. of perchloric acid are added, the cover of the beaker is raised with a glass hook, and the solution is evaporated to dryness on a hot plate at a temperature not higher than 350° C.

Twenty milliliters of anhydrous *n*-butyl alcohol and 0.2 ml. of perchloric acid are added to the mixed perchlorates of potassium, sodium, and lithium, the solution is heated to boiling, and 8 ml. of a 20% solution of hydrogen chloride in *n*-butyl alcohol are added, the first milliliter dropwise, while the mixture is constantly stirred. After the solution has cooled to room temperature the precipitate (consisting of sodium chloride and a mixture of potassium chloride and potassium perchlorate) is collected on a glass filter or Gooch crucible and washed about eight times with a 6% solution of hydrogen chloride in *n*-butyl alcohol. The crucible is held if a determination of sodium and/or potassium is also required.

The filtrate and washings, which have been received in a 250-ml. beaker, are diluted with one third their volume of water, thus forming two layers, the lithium chloride and perchlorate being contained in the water layer. The whole is evaporated on the water bath in such a way as to avoid condensation on the upper part of the beaker. (This can easily be done by immersing the beaker through a hole in an asbestos or metal plate into an open metal cylinder which is heated by the steam of the water bath.)

When completely dry, 10 ml. of water, 5 ml. of nitric, 3 ml. of perchloric, and 1 ml. of sulfuric acids are added and the whole is evaporated on a hot plate to strong fumes of sulfuric acid. (This treatment usually is sufficient to destroy any brown coloration due to organic matter; if not, a few drops of nitric acid should be added and the fuming resumed.) The excess sulfuric acid is finally fumed off, 15 ml. of water are added to the cool beaker, and the solution is heated to boiling, transferred to a previously ignited and weighed platinum dish, and evaporated as far as possible on the water bath. The platinum dish is now heated with a small Bunsen flame until all acid has been expelled, finally for one minute at a dull red heat. When cool, the platinum dish is weighed; the increase in weight is lithium sulfate.

For extreme accuracy, the analyst should determine a correction for the solubility of sodium chloride, potassium chloride, and potassium perchlorate in the 6% solution of hydrogen chloride in *n*-butyl alcohol and a blank for all the reagents used. The correction usually is less than 1 mg.

Should determination of sodium and/or potassium be also required, the residue is dissolved on the glass filter or Gooch crucible in hot water and sodium separated from potassium according to standard methods.

**Table VIII. Separation of Lithium from Sodium and Potassium**

KCl Taken Gram	NaCl Taken Gram	LiCl Taken Equivalent to Li <sub>2</sub> SO <sub>4</sub> Gram	Li <sub>2</sub> SO <sub>4</sub> Found Gram
0.0250	0.0250	0.0735	0.0739
0.0500	0.0500	0.0735	0.0736
0.0500	0.0500	0.1470	0.1476
0.0500	0.1000	0.1470	0.1475
0.1000	0.0500	0.1470	0.1472
0.1000	0.2500	0.1470	0.1477
0.2500	0.1000	0.1470	0.1476
0.5000	0.5000	0.1470	0.1476
0.2500	0.2500	0.2205	0.2210
0.2500	0.2500	0.4410	0.4412

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# Adaptation of a Waring Blendor for Continuous Emulsification

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IN 1939, Davis (2) pointed out the advantages of the Waring Blendor for extracting plant and animal tissues. Since that time this type of machine has been employed by many laboratories, usually in connection with extractive and analytical procedures. It was recognized that the utility of the Blendor was limited by the characteristics of the original container and that the usefulness of the device would be increased if blending vessels of different capacities and shapes were available for operation on the same motor shaft as the original container. Davis devised such vessels but did not describe the method of their construction. Benne (1) extended this idea and reported upon the preparation and use of three different kinds of blending vessels which possessed advantages over the commercial container for certain purposes.

This paper reports an adaptation of the Waring Blendor that has been used successfully for the preparation of emulsions by a continuous process. Since small blending vessels which operate on the base of the Blendor are advantageous for certain analytical extractive procedures and are essential to the continuous emulsification process reported in this paper, a convenient method for their construction is described.

## CONSTRUCTION OF SMALL BLENDING VESSELS

The small blending vessels shown in Figure 1 were constructed by making holes 0.75 inch in diameter in the bottom of the glass bottles shown and mounting therein commercial blending assemblies which are obtainable on the market. Appropriate alterations in the blades of these assemblies were made where necessary, so that they could be accommodated by the vessels of smaller size. The holes through the glass were made by penetration with a small steel drill, followed by enlargement of this hole to the proper size with round files of different diameters. Turpentine used on the files during this operation greatly facilitated cutting.

As can be seen, it was necessary to prepare wooden adapters to hold the improvised vessels in position on the motor shaft. Four long, slender screws were inserted into the wooden base, one at the center of each side of the bottle for Nos. 3 and 4, to prevent rotation with the motor shaft. For No. 2 the long screws were

replaced by narrow strips of spring steel which were fastened to the base with small screws. To act as shock absorbers, a piece of small-bore rubber tubing of appropriate length was slipped over each of the long screws or steel strips, and a piece of rubber gasket sheeting, shaped to fit the container seat, was placed beneath the wooden adapter.

Although the method described above for making holes through glass vessels is simple and involves no unusual equipment, it is laborious and time-consuming. The authors were interested therefore in the method used by Huddleson (3) for this purpose.

This investigator had prepared in the college machine shop a glass-cutting tool similar to those pictured in Figure 2. This tool consisted of a brass tube 0.75 inch in diameter with walls approximately 0.0625 inch thick, attached to a solid steel rod, the upper end of which was reduced to about 0.375 inch in diameter, so that it could be accommodated by the chuck of an electrically driven drill press. The tool is employed by clamping it in the chuck of the press, adding Carborundum powder and a small amount of turpentine to the glass where the hole is desired, lowering the tool into the mass of abrasive, and using it as a core drill against the glass. Two deep nicks in the lower end of the tube and a hole in the wall above permit the turpentine and

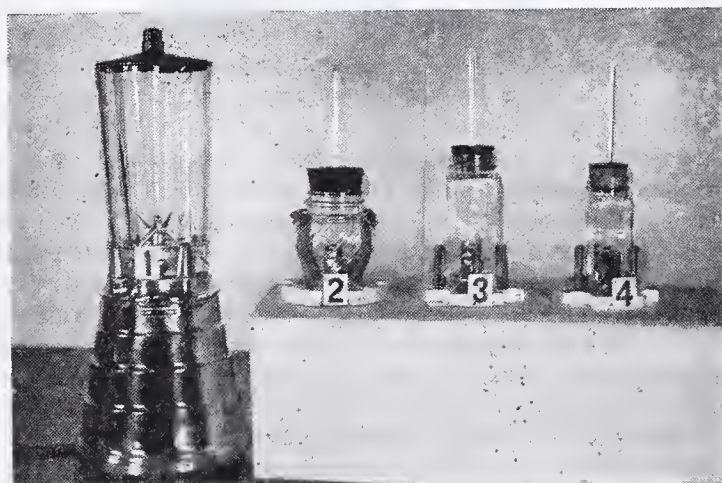


Figure 1. Waring Blendor and Three Containers

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abrasive to flow back into the path of the cutting tool. The mixture of turpentine and abrasive is retained in the proper place on a flat or convex surface by molding a reservoir from modeling clay against the glass around the spot to be drilled.

This method of cutting round holes through glass surfaces is satisfactory and reduces to a few minutes the time for a task which formerly required hours. Adapting the blending assemblies to vessels of different size and preparing adapters for holding them in position on the Blendor base must be carried out as before.

#### ADAPTATION FOR CONTINUOUS EMULSIFICATION

The Waring Blendor offers a simple and effective mechanical device for preparing paraffin wax-in-water emulsions. A small experimental amount of an emulsion of this type can be prepared by placing hot water and an appropriate emulsifying agent in the blending vessel, starting the motor, and adding the molten wax dropwise. By controlling the temperature of the material and the time of agitation, reproducible conditions can be readily maintained. It was noted that better emulsions could be obtained with an improvised vessel similar to No. 3, Figure 1, than with the container supplied with the machine. When the small vessel was used for this purpose a one-hole rubber stopper was inserted in the top before starting agitation and the molten wax added through the hole.



Figure 2. Tools for Cutting Round Holes through Glass Surfaces

The greater efficiency of the smaller vessel is probably due to its square corners which act as swirl arrestors and to decreased clearance between the blades of the blending assembly and the sides of the container, since these features should tend to increase the force of impact of the ingredients against the walls. This was important with the emulsion systems used by the authors, because their work required that a minimum adequate amount of emulsifying agent be used, and very effective mechanical agitation was required to obtain stable emulsions. Ordinary laboratory stirrers were inadequate for this purpose, whereas agitation with a Waring Blendor using one of the small containers produced emulsions of satisfactory stability. The length of the agitation period necessary to achieve the desired state of

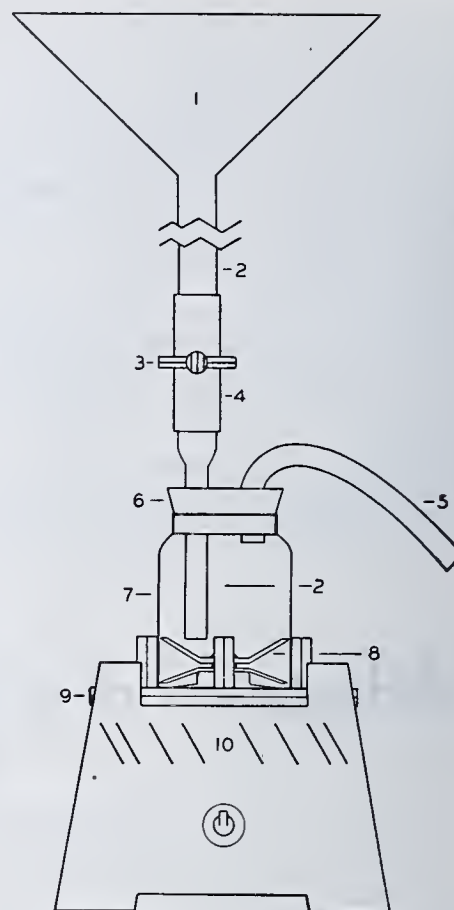


Figure 3. Diagram of Blendor

- |                        |                         |
|------------------------|-------------------------|
| 1. Funnel              | 6. Rubber stopper       |
| 2. Glass tubing intake | 7. Blending bottle      |
| 3. Screw clamp         | 8. Blade assembly       |
| 4. Rubber tubing       | 9. Bottle support       |
| 5. Outlet              | 10. Waring Blendor base |

emulsification is variable, since in any emulsion system the amount of mechanical agitation required will depend upon the formula used, one of the most important factors being the amount and effectiveness of the emulsifying agent added.

For preparing larger amounts of emulsion a Waring Blendor and one of the smaller blending vessels were arranged for a continuous-flow process as shown by the schematic diagram in Figure 3.

The ingredients of the emulsion flow by gravity from the elevated funnel, which acts as a reservoir, through the glass connecting tube directly onto the blending assembly. The rate of flow is controlled by means of a screw clamp on the rubber tubing connecting the glass tube and the funnel. It was found advisable to heat the ingredients and to mix them with a laboratory stirrer before introducing them into the continuous system. Introduction of the ingredients separately was tried, but afforded no advantages over the other procedure. As will be noted from the figure, the completed emulsion leaves the blending vessel through the top; hence, the cup is entirely full during the emulsifying process. This arrangement effectively prevents the beating of air into the emulsion during its preparation. Using two such Blendor systems simultaneously as much as 16 gallons of concentrated emulsion, equivalent to 160 gallons of diluted spray material, have been prepared in 8 hours in this laboratory.

After the premixing process the diameters of the droplets of the dispersed phase in a standard wax emulsion varied from 1 to 30 microns, with the majority about 5 microns; however, after having been passed through the Blendor system there were very few droplets larger than 2 microns in diameter, and most of them were about 1 micron. Hence, in a laboratory not equipped with a colloid mill the continuous Blendor system offers an inexpensive and effective means of emulsification with a capacity adequate for many experimental purposes.



It is possible that the efficiency of this method of emulsification could be increased by varying the design of the apparatus. For example, greater efficiency might result by reducing the space between the blending assembly and the top of the cup or by introducing the ingredients below the blades, or through the side wall directly onto the blades. A steam- or electrically heated funnel for the reservoir should prove advantageous. Certain of these features are under investigation.

## Determining Hydrogen in Gases With a Thermal-Conductivity Apparatus

G. A. WEBB<sup>1</sup> AND G. S. BLACK, Mellon Institute, Pittsburgh, Pa.

A thermal-conductivity cell was used to determine the hydrogen content of the exhaust gases from a catalytic-dehydrogenation unit. These exhaust gases consisted principally of hydrogen, but contained several other components such as methane, ethylene, carbon dioxide, etc., totaling not more than 30 to 35% by volume. The latter were grouped together and considered as one component to form a simple two-component system. It was determined that, if the cell is calibrated with simple mixtures similar to the gases to be analyzed, an accuracy in the hydrogen analysis of  $\pm 1.5\%$  can be obtained. The method is satisfactory for appraisal of dehydrogenation catalysts.

IN MANY catalytic operations the activity of a catalyst can often be evaluated by analyzing the exit gas. When the exit gas is analyzed by conventional absorption methods, the analytical procedure is time-consuming and large samples are required. Moreover, the analysis of this large sample indicates an average activity over the sampling period which is misleading when dealing with catalysts of short life.

This problem was encountered in a study of the dehydrogenation of ethylbenzene. The actual conversion to styrene was determined by a bromine number analysis of the liquid condensate but it was desirable, especially to give information as to the specificity of the catalysts, to know the hydrogen content of the noncondensable gas.

In this broad catalytic program in which catalysts of widely varied activity were tested, it was found that the average values given by conventional gas absorption analyses were inadequate, and a method capable of giving instantaneous gas compositions was indicated.

The analysis of the exhaust gases by thermal-conductivity means was considered as a solution of this problem. This method is well known for applications involving a binary gaseous mixture, and for more complex mixtures wherein certain components are present in constant amount or can be removed by chemical or physical means (2, 3). The obvious disadvantage to its use in this application is the number of components present in the exit gases. A series of typical analyses of the exhaust gases from a unit evaluating the catalytic dehydrogenation of ethylbenzene to styrene is given in Table I. The results were obtained by the usual volumetric methods for gas analysis.

These exhaust gases consist principally of hydrogen with lesser amounts of other gases. Since the thermal conductivities of the minor components (carbon dioxide, ethylene, nitrogen, etc.) are practically identical when compared to hydrogen, the hydrogen value given by thermal conductivity is not affected by the distribution of the minor components. This is shown by the figures presented in Table II which were calculated on the assumption

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PUBLISHED with permission of the Director of the Experiment Station as Journal Article No. 704 (n.s.). This research was supported in part by the Horace H. Rackham Research Endowment of the Michigan State College of Agriculture and Applied Science for studies on the industrial utilization of agricultural products.

that the conductivity is proportional to the molecular concentration. From these figures, it is apparent that the exhaust gases can be handled as a two-component system.

### APPARATUS

The apparatus consisted of an air thermostat, a milliammeter, a storage battery, and a Minter diffusion-type thermal-conductivity cell (a development of Clarke C. Minter, available through the Gow-Mac Instrument Co., 22 Lawrence St., Newark 5, N. J.).

The Minter cell is made from a machined, 2-inch brass cube which has four hollow cylindrical spaces in it. Each space contains a small, glass-coated, platinum resistance wire. The wires are connected in a conventional Wheatstone-bridge circuit and

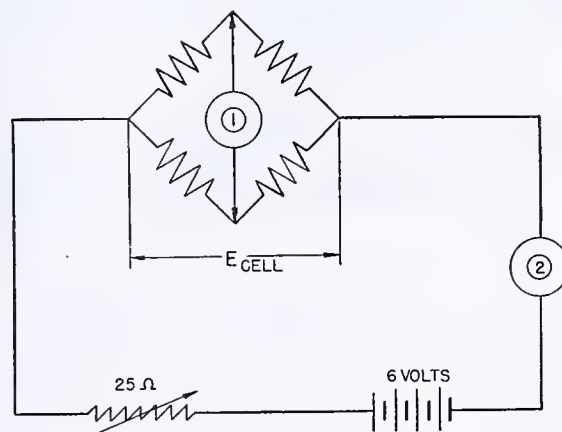


Figure 1. Electrical Circuit for Cell

1. Potentiometer, e
2. Cell current meter, I cell

Table I. Analysis of Miscellaneous Exit Gases from Catalytic Dehydrogenation of Ethylbenzene

CO <sub>2</sub>	C <sub>3</sub> +	C <sub>2</sub> H <sub>4</sub>	O <sub>2</sub>	CO	H <sub>2</sub>	Paraffins <sup>a</sup>	N <sub>2</sub>
Per Cent by Volume							
10.6	0.2	4.2	0.8	1.4	74.0	5.8	3.0
0.8	0.0	7.3	0.7	1.0	78.0	9.2	3.0
18.0	0.2	12.8	0.6	0.4	54.8	10.8	2.4
18.8	0.0	11.2	0.4	0.2	59.6	7.6	2.2
11.2	1.8	5.0	0.4	0.8	70.2	1.6	9.0
7.0	0.2	11.8	0.6	0.2	65.8	12.4	2.0
18.1	0.1	7.9	0.6	0.4	61.6	7.8	3.5
13.3	0.0	2.0	0.0	1.8	75.6	7.3	0.0

<sup>a</sup> Average paraffin index 1.3.

Table II. Calculated Thermal Conductivity of Various Gas Mixtures

Gas Composition (Per Cent by Volume)						Thermal Conductivity, K cal./cm. sec. ° C. $\times 10^{-5}$
CH <sub>4</sub>	C <sub>2</sub> H <sub>6</sub>	C <sub>2</sub> H <sub>4</sub>	CO <sub>2</sub>	N <sub>2</sub>	H <sub>2</sub>	
5	5	5	5	5	75	31.68
12	13	0	0	0	75	31.86
0	0	5	20	0	75	31.34
15	0	0	5	5	75	31.98

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are heated by passing through the bridge a small direct current of about 0.5 ampere. The internal construction of the cell is such that one pair of opposite resistances in the bridge is exposed to a standard gas, and the other pair is exposed to the unknown gas being compared with the standard. The brass cell is maintained at 30° C., and since this temperature is considerably lower than the temperatures of the resistance wires, heat is lost from the wires to the cell block by the conduction of gases.

Resistance measurements indicate the rate of heat loss from the wires and measure the thermal conductivity of the gases which surround the wires. When the thermal conductivities of the two gases being compared are different, owing to differences in compositions, the resistance wires are cooled at proportionally different rates and their resistance is changed, producing an electrical unbalance in the bridge circuit. This unbalanced condition is read by a potentiometer, and these readings are a function of the gas compositions. The circuit used in this work is shown in Figure 1.

### EXPERIMENT

Various methods have been used in the calibration of this type of conductivity cell (1), dynamic methods probably having been preferred because of the advantage of greater accuracy. However, in the present application a direct method was used.

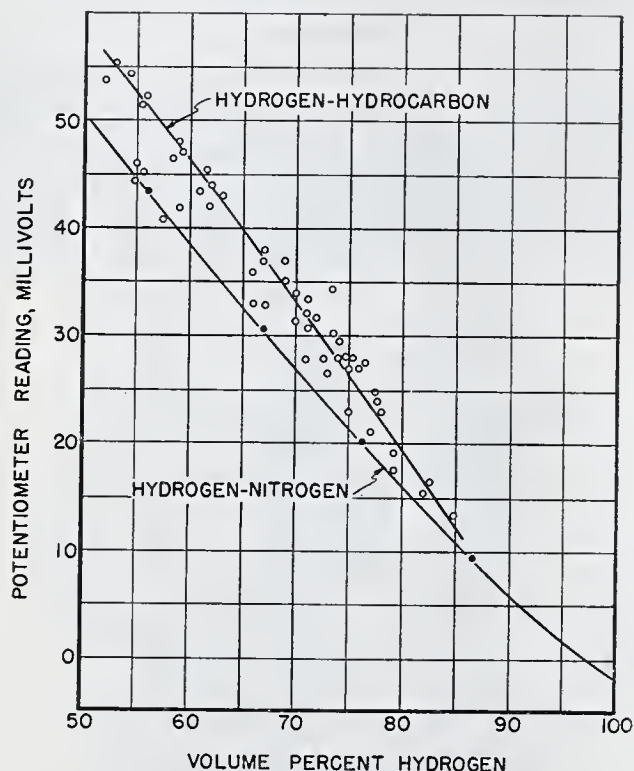


Figure 2. Correlation of Cell Readings with Hydrogen Content

Mixtures of hydrogen and nitrogen of various compositions were prepared and passed through the cell for a period of one hour. Cylinder hydrogen was used as the standard gas. The composition of an average sample of these calibration mixtures was determined by the usual method of volumetric gas analysis. The average cell-reading obtained on the potentiometer was plotted against the composition of these mixtures and is shown in Figure 2.

The choice of a nitrogen-hydrogen mixture for the calibration of the cell for establishing a curve of conductivity versus hydrogen content was based upon the magnitude of the thermal conductivity of nitrogen, which lies about halfway between methane and ethylene. Probably a better calibration mixture would have been carbon dioxide-nitrogen and hydrogen.

The data plotted as the upper curve in Figure 2 show the potentiometer readings for a number of exhaust-gas compositions, obtained by separate chemical analyses of the exhaust gases from a number of dehydrogenation runs. The difference between the actual hydrogen content of the exhaust gases and the value obtained from a nitrogen-hydrogen calibration curve is shown in Figure 2.

### DISCUSSION

Examination of Figure 2 shows that the thermal conductivity method can be used to determine the hydrogen content of mixed exit gases from a catalytic dehydrogenation unit, the accuracy being dependent upon the calibration curve used. In the present case the use of a nitrogen-hydrogen calibration curve gave hydrogen figures which were about 4% low. However, using the calibration curve established by the various mixtures themselves an accuracy of about  $\pm 1.5\%$  was obtained.

Obviously, maximum accuracy results when the calibrating mixture is identical with the actual composition to be evaluated, but for the quick appraisal of catalysts it is evident that a calibration with a simple nitrogen-hydrogen mixture will in most cases be satisfactory.

In addition, the method can be used in a comparative manner, without a calibration curve, by evaluating the exhaust gas from a catalyst of known activity, and rating others on the basis of the difference in the thermal conductivity values of the exhaust gases obtained.

### ACKNOWLEDGMENT

The authors are indebted to Miss B. Zehner and to E. E. Fleck, who determined most of the numerical data presented, and to C. C. Minter for his gift of the conductivity cell.

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## Facilitating Reading of Volumes in Determinations of Moisture by Distillation

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IN MOISTURE tests by distillation methods it is occasionally difficult to read accurately the volume of water that has distilled, owing to the collection of small drops on the sides of the tube or to peculiar surface effects at the interface of the liquids (1).

It has been found that the addition of a small amount of the solvent containing a wetting agent will eliminate sticking of droplets and aid in the formation of a clear meniscus. Only two agents were tried, Tween 20, made by the Atlas Powder Co., and sodium lauryl sulfate. Both were effective, and it appears probable that other products of the same type would serve equally well in the same capacity.

The amount of wetting agent used must be of the magnitude of one drop or equivalent amount of solid in 20 or 25 ml. of solvent and only 2 or 3 ml. are necessary for one determination. Use of a larger amount tends to cause formation of a dispersion. The solution may be placed in the receiving tube before the determination is begun, or added just before the final reading is taken.

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# NOTES ON ANALYTICAL PROCEDURE

## Preparation of Standard Cerate Solutions from Cerium Titration Residues

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COMPARED with permanganate and bichromate salts, cerium compounds are rare. Therefore, the procedure for recovery of volumetric cerium described below was developed and has been used in this laboratory for several months:

**PROCEDURE.** Titration residues containing cerium are collected in a suitable vessel. After a sufficient amount of residue has accumulated, an excess of saturated oxalic acid solution is added, followed by sufficient concentrated sodium hydroxide to complete the precipitation of  $\text{Ce}_2(\text{C}_2\text{O}_4)_3 \cdot 9\text{H}_2\text{O}$  (3). The solution should remain distinctly acidic (0.5 to 1.0  $M$  in  $\text{H}^+$ ) to prevent the precipitation of alkaline earths, iron, and other alkali-insoluble ions (4). After the precipitate settles, the major portion of the supernatant liquid is poured off, and the precipitate is transferred to a Büchner filter. If manganese or uranyl ions are known to be present in the solution, the precipitate is redissolved in the smallest possible volume of concentrated hydrochloric acid. Cerous oxalate is then reprecipitated by dilution to twenty times the volume of hydrochloric acid used. If neither manganese nor uranyl ions are present, the precipitate is washed on the Büchner with 0.1  $M$  hydrochloric acid, followed by several water washes, and the material is dried with acetone or alcohol. It is then transferred to a shallow evaporating dish and heated with the yellow-tipped flame of a Méker burner to ignite the oxalate to ceric oxide (1). The mass presently turns dark gray in color, and heating is continued with stirring until the gray color is supplanted by the buff of ceric oxide. This operation requires 10 to 15 minutes. The ceric oxide is now ready for use, and may be dissolved in sulfuric or nitric acid and made up to standard. The oxide should be allowed to digest with the concentrated acid for one hour below the boiling point before dilution.

Perchlorato cerate may be prepared by suspending the oxalate in 4 to 6  $M$  perchloric acid and electrolytically oxidizing the material, following the method of Smith, Frank, and Kott (5).

If phosphoric acid is present in the titration residues, the recovery is impractical, because excessive amounts of sodium hydroxide are required to neutralize the high acid concentration necessary when tetravalent cerium is used with phosphate, and because ceric phosphate is insoluble in acid solutions.

**DISCUSSION.** The procedure has been tested on solutions containing  $\text{Fe}^{+++}$ ,  $\text{Mn}^{++}$ ,  $(\text{UO}_2)^{++}$ ,  $\text{V}^{++++}$ ,  $\text{Mo}^{++++}$ ,  $\text{Cr}^{+++}$ ,  $\text{Cu}^{++}$ ,  $\text{Co}^{++}$ ,  $\text{As}^{++++}$ , and  $\text{Sb}^{++++}$ . Small amounts of  $\text{Mn}^{++}$  (2) and  $(\text{UO}_2)^{++}$  (2) are precipitated along with the cerous oxalate, and must be removed by the hydrochloric acid treatment if they would be likely to interfere in the use of the standard solution. In no other case was the precipitate found to be contaminated.

The percentage recovery of cerium following the procedure described is dependent upon several factors: the concentration of the cerium in the residues; the final acidity of the mother liquor; the nature of the contaminants, manganese and uranyl ion lowering the recovery since a reprecipitation is necessary; the completeness of conversion of the oxalate to the dioxide; and the temperature to which the oxide is heated during the ignition, high temperatures resulting in the formation of some refractory, insoluble ceric oxide. Careful control of the conditions, however, is unwarranted, since the percentage recovery under ordinary conditions seldom falls below 95%.

**SUMMARY.** Sulfato, nitrate, and perchlorato cerate solutions may be prepared from titration residues containing cerium. The procedure is simple and inexpensive, allowing a recovery of

95%. The only common interferences with the recovery are scandium, yttrium, and the other rare earths and phosphate ion.

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## Crucible Holder for Use with Rubber Extraction Apparatus

WM. E. BOYD, Inspection Board of United Kingdom and Canada, Nobel, Ontario, Canada

TO ELIMINATE the difficulty of attaching sintered-glass crucibles by wires or wire baskets to the condensers of Underwriters' rubber extraction apparatus, the following method was devised.

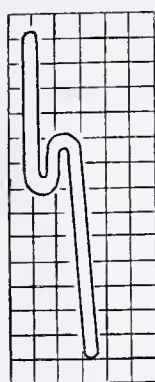


Figure 1

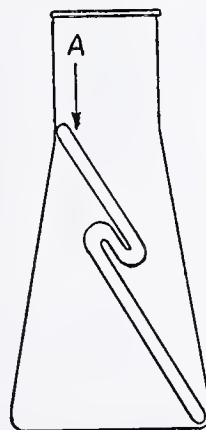


Figure 2

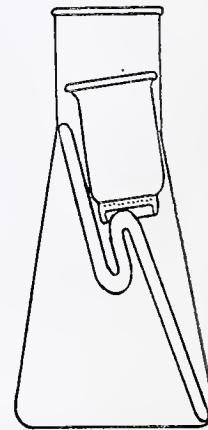


Figure 3

A 5-mm. glass rod is bent as shown in Figure 1, where spaces between parallel lines represent 1-cm. distances. The bent glass rod, dropped into a 400-ml. flask, assumes the position shown in Figure 2. When a sintered-glass crucible is then inserted, the bottom edge exerts a slight pressure on the rod at A, Figure 2, causing it to swing around and thus support the crucible as shown in Figure 3.

After extraction the crucible is easily lifted out with metal forceps and the rod washed with a small amount of the extracting solvent.



# A Device to Relieve Bumpy Distillations

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**T**O PREVENT bumping during distillation some mechanism is necessary to disperse the heat applied and to agitate the fluid to be distilled. The device herein described has proved extremely effective. Its principle is basically the same as that involved in a Cottrell (1) pump for use in the determination of boiling points of solution. However, the apparatus was devised in the absence of any knowledge of the Cottrell pump.

A glass tube is bent in a U-form, with one arm somewhat shorter than the other, inverted, and introduced into the distilling flask (A). The U-tube should be long enough to extend from the bottom of the flask for a short distance into the neck. Its length thus serves to hold it in the upright position. If for any reason a shorter U-tube is desired, a glass rod may be welded to it to hold it upright (B). The short arm of the U should be just above the level of the fluid in the flask, while the long arm should rest on the bottom of the flask directly above the source of heat. No attempt should be made to have the end of the long arm fit uniformly around its rim on the bottom of the flask, although no ill effects from a close fit are anticipated. Obviously it should not fit the bottom of the flask so tightly as to prevent the distilling fluid from passing under it. If a large flask is used for distilling a large quantity of fluid, two or three U-tubes should be added, the short arm of one of which is just above fluid level at the start of distillation. The short arms of the other U-tubes should be of different lengths, and below fluid level.

As the fluid reaches the boiling point, the heat will cause fluid and vapor to rise to the top of the tube. Then fluid drops descend in the short arm of the tube by a combination of gravity and the force of the applied heat. In this way agitation is produced and the superheated fluid at the bottom of the flask is partly carried away. When a series of tubes is used, one or more have their short arms above the level of the fluid at all times and as the fluid level drops in the flask, others come in to play. Some advantage is secured, even though both ends

are submersed in the fluid, from the circulation of vapor bubbles and fluid through the inverted U-tube. At times it is an advantage to have the long end of the tube flared slightly in the fashion of a funnel (see diagram). The diameter of the glass tube can vary somewhat; an inside diameter of 2 to 3 mm. has been found effective. Modifications can be adapted to meet many needs—particles in the distilling fluid, viscosity, etc.

If the presence of metal in the distilling flask is not objectionable a polished brass or aluminum rod, 3 to 4 mm. in diameter, may take the place of glass.

Many of the authors' friends have used the inverted U-tube method to control bumping, and recently its use has spread to other universities and laboratories.

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# Take-Off for Recovering Solvent from Rubber Extraction Apparatus

WM. E. BOYD, Inspection Board of United Kingdom and Canada,  
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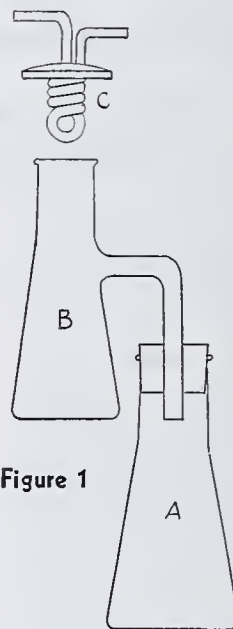


Figure 1

**I**NSTEAD of allowing solvent (carbon tetrachloride) to boil away after extracting material in the Underwriters' rubber extraction apparatus, the following simple take-off was devised.

To a flask of sufficiently wide mouth to accommodate the condenser is attached a piece of glass tubing having an outside diameter of at least 10 cm. and bent in the form illustrated in Figure 1. After extraction has been effected in the extraction flask, A, the condenser, C, is raised, the extraction thimble is removed, the bent-arm flask, B, is connected with the extraction flask, using a cork stopper, and the condenser is placed in the mouth of the bent-arm flask. The solvent is then boiled out of the extraction flask and the condensate caught in the bent-arm flask, care being taken to avoid excessive heating or drying of the solute in the extraction flask.

Solvent recovery is excellent and residual material in A is in no way contaminated for further chemical treatment.

## NEW EQUIPMENT and BOOK REVIEW

### Fluoride Tester

The Harrold fluoride tester, a test instrument devised for rapid analysis of a contaminated atmosphere when hydrofluoric acid, sodium fluorides, or calcium fluorides are present, is announced by the Production Equipment Co., 6432 Cass Ave., Detroit 2, Mich. Developed as an aid to safety engineers for the analysis of welding atmospheres and for use in light metal foundries, the complete test kit weighs less than 5 pounds. The air is drawn past a calibrated test paper, which instantly changes color if fluorides are present. Test papers are guaranteed to be stable for 3 months.

### Geiger-Counter Spectrometer

A new x-ray instrument, the Geiger-counter spectrometer, was unveiled publicly at the National Metal Congress and Exposition, held October 16 to 20 in Cleveland, Ohio. A product of the North American Philips Co., Inc., New York, it utilizes a Geiger-Müller tube to measure the intensity and position of interference lines encountered in x-ray diffraction analysis work.

**Laboratory Guide in Chemistry.** Joseph H. Roe. 192 pages. C. V. Mosby Co., St. Louis, Mo., 1944. Price, \$1.00.

Experiments in this laboratory guide, which is bound in a plastic loose-leaf binding, are designed to accompany the author's textbook "Principles of Chemistry", and were incorporated in former editions of the text. Forty exercises are presented, five of them prepared for this edition.



## Applicability of Newer Physical Methods for Hydrocarbon Analyses

W. J. SWEENEY, Esso Laboratories, Elizabeth, N. J.

Some of the newer physical methods are finding wide application in hydrocarbon analyses. Many highly complex problems are greatly simplified by a judicious use of these methods. The author summarizes briefly the advantages resulting therefrom in the analysis of petroleum products.

OVER the past several years great progress has been made in applying the principles of physics to methods of analysis for hydrocarbons. For security reasons, very little of this work has yet been published. The purpose of the present article is merely to indicate the applicability of this published information to the needs of research in the petroleum industry. A short bibliography is appended for more detailed references on the subject.

Experience has shown that it is profitable to take analytical work out of the routine class and to consider it as a vital part of petroleum research, development, and manufacturing. The physicists and the manufacturers of optical and electrical instruments have opened up a new field in this class of analytical work. This development is still in its infancy; but it has made such strides as to warrant confidence of its future indispensability in serving the petroleum industry in laboratory research as much as other physical methods have helped in oil field production. This new analytical technique will not replace older methods entirely. It has already been amply demonstrated by experience

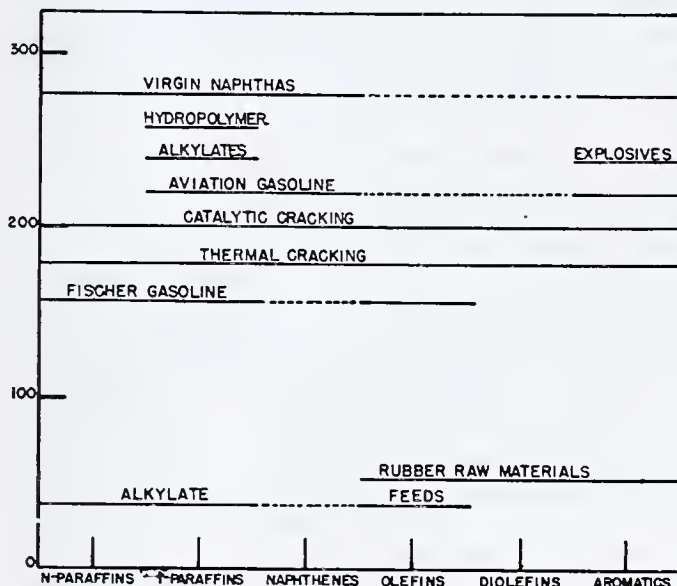


Figure 2. Classes of Hydrocarbons

that all analytical methods, new and old, are less competitive and more complementary when they are most efficiently applied. Moreover, in the present state of the art, it is not always possible to decide beyond question which instrument is best for any purpose. As an example of the variety of equipment for which a modern petroleum research laboratory can have full-time use, a partial list of the physical instruments currently used by the Esso Laboratories-Research Division of the Standard Oil Development Company is as follows:

- 20 Fractional distillation columns (Fenske packing)
- 6 Podbielniak Hyd-Robot columns
- 2 Podbielniak manually operated columns
- 1 Gaertner infrared spectrograph
- 1 Beckman infrared spectrophotometer
- 1 Perkin-Elmer infrared spectrograph
- 2 Beckman ultraviolet spectrophotometers
- 1 ARL-Dietert emission spectrograph
- 1 Westinghouse mass spectrograph
- 1 Raman spectrograph (accessible but not owned)

This list does not, of course, include all the conventional chemical equipment that was used prior to the introduction of the newer techniques and is still being used concurrently with them.

### PROBLEMS OF PETROLEUM ANALYSIS

A satisfactory way to become oriented as to the interdependence of these methods is to consider the complexity of petroleum and of the problems of its analysis. To illustrate this point, in Figure 1 the isomers of normal paraffins, isoparaffins, naphthenes,

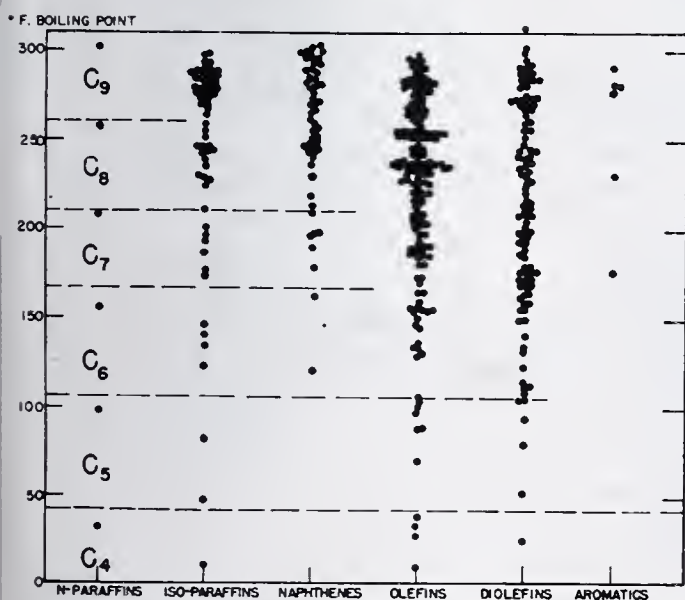


Figure 1. Distribution of Hydrocarbon Groups by Boiling Point



Table I. Hydrocarbons Found in Typical Crudes

(A.P.I. Project 6)

Paraffins, 40–102° C.	Naphthenes, 40–102° C.	Aromatics, 80–160° C.
2,2-Dimethylbutane	Cyclopentane	Benzene
2,3-Dimethylbutane	Methylcyclopentane	Toluene
2-Methylpentane	Cyclohexane	Ethylbenzene
3-Methylpentane	1,1-Dimethylcyclopentane	<i>p</i> -Xylene
<i>n</i> -Hexane	1,3-Dimethylcyclopentane (T)	<i>m</i> -Xylene
2,2-Dimethylpentane	1,2-Dimethylcyclopentane (T)	<i>o</i> -Xylene
2,4-Dimethylpentane	Methylcyclohexane	Isopropylbenzene
2,3-Dimethylpentane		<i>n</i> -propylbenzene
2-Methylhexane		
3-Methylhexane		
<i>n</i> -Heptane		
	Total Possible Compounds	
15	52	8

olefins, diolefins, and aromatics are grouped according to boiling point, up to and including hydrocarbons of 9 carbon atoms. The complexity of the problem is readily seen, when considering the possible mixtures that may exist with boiling points above 100° F. In Figure 2 an attempt has been made to show diagrammatically the classes of hydrocarbons in various types of materials and products, with particular reference to petroleum, for which complete analyses are highly useful in manufacturing and refining control. The full complexity of this problem can also be visualized by superimposing this diagram on that of Figure 1.

Fortunately, however, in most cases all the possible components are not present, and in practice the problem is not so complex as at first appears. Nature has been helpful. A good example of this is shown in Table I. Here are listed the paraffins and naphthenes boiling between 40° and 102° C. (104° and 216° F.), and the aromatics boiling below 160° C. (320° F.) which were found by Rossini and his co-workers (4) in virgin naphthas occurring naturally in petroleum crude oil. Of the paraffins, 11 out of the possible 15 have been identified. Only 7 naphthenes are indicated to be present in appreciable quantities out of the possible 52. On the other hand, all the possible aromatics were found to be present.

In general, the newer methods of analysis cannot be applied directly to mixtures containing a large number of components. For this reason, it is necessary to "divide to conquer". To obtain accurate and reliable results, the number of components should be decreased to about 8, and preferably fewer, by employing all available means of separation, such as fractional distillation.

As an illustration of what can be done along these lines, Figure 3 shows a combination method for the analysis of a complete C<sub>4</sub>-hydrocarbon cut. The butadiene content can be determined directly by ultraviolet absorption, but the remaining compounds are analyzed by infrared absorption, only after making appropriate separations to reduce the complexity, and that entails the removal of the butadiene. The amount of *n*-butane and isobutane is determined on a part of the sample from which the olefins have been removed with bromine. Infrared measurements are then made on another part of the total sample; these measurements are corrected for the amount of *n*-butane and isobutane previously found; and the amount of each of the C<sub>4</sub>-olefins is then calculated.

By using this method, the required calculations are greatly reduced as compared to those required when the analysis is made by infrared alone on the total sample. A preliminary fractional distillation as in the Podbielniak Hyd-Robot (5) also contributes to the accuracy of this type of analysis by removing C<sub>3</sub> and C<sub>5</sub> hydrocarbons, which definitely interfere with the infrared analysis. It is not necessarily true that the method here outlined is the best for analyzing the C<sub>4</sub> mixture; but it is true that the best method will involve an analogous system of simplifying short cuts based on good judgment. Just as the expert golfer uses a variety of golf clubs, an expert in hydrocarbon analysis will use the combination of instruments best suited to the particular job.

While these newer methods have resulted both in shortening the time required for certain analyses and in permitting other analyses to be done which were not previously possible, it is not therefore to be inferred that earlier workers were wholly in the dark. The "old-fashioned" chemical and physical methods have been and are being used to good effect in obtaining analyses, both as to type and as to specific hydrocarbons, of many materials important to the war effort—for example, the components of aviation gasoline and of synthetic rubber. There will always be a need for the older methods. Such techniques as distillation, fractional or azeotropic, crystallization, solvent extraction, bromination, or other chemical methods of separation and identification, will be useful and necessary parts of the analytical stock in trade. In particular, they will be used for type analyses on complex mixtures, as a guide to the choice of optical methods and of the proper individual hydrocarbons to be prepared in utmost purity for background standards in spectral analysis. It is obviously impossible to stock a "bank" with standard samples of all conceivable hydrocarbons. A laboratory working in a

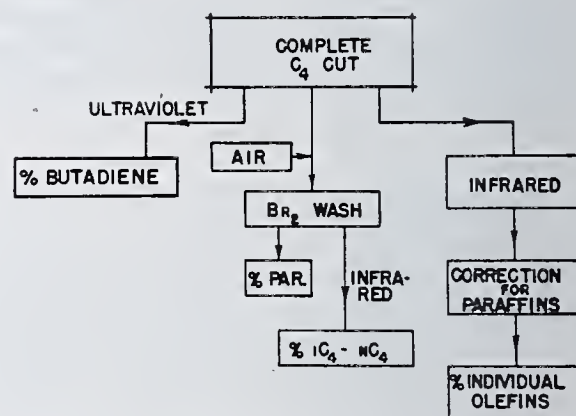
Figure 3. Combination of Methods for Analysis of C<sub>4</sub> Cut

Table II. Hydrocarbons Required for Spectrometer Calibrations (A.P.I. Project 46)

Paraffins			
C <sub>1</sub> -C <sub>4</sub> , all isomers	5		
C <sub>5</sub> , all isomers	3		
C <sub>6</sub> , all isomers	5		
C <sub>7</sub> , all isomers	9		
C <sub>8</sub> , liquid isomers	17		
C <sub>9</sub> , lower isomers	5		
C <sub>9</sub> -C <sub>10</sub> , normal compounds	2		46
Aromatics			
C <sub>6</sub> -C <sub>8</sub> , all isomers	6		
C <sub>9</sub> , all isomers	8		14
Alkyl cyclopentanes			
C <sub>6</sub> -C <sub>7</sub> , known isomers	7		
C <sub>8</sub> , known isomers	11		18
Alkyl cyclohexanes			
C <sub>6</sub> -C <sub>8</sub> , all isomers	10		10
Aliphatic olefins			
C <sub>2</sub> -C <sub>5</sub> , all isomers	12		
C <sub>6</sub> , below 50° C.	1		13
Aliphatic diolefins			
C <sub>3</sub> -C <sub>4</sub> , allene, butadiene	2		
C <sub>6</sub> , isoprene; <i>cis</i> -1,3-, <i>trans</i> -1,3-, 1,4-, 2,3-	5		7
Acetylenes			
C <sub>2</sub> -C <sub>4</sub> , simple isomers	4		4
Cyclic olefins			
C <sub>5</sub> , cyclopentene	1		1
Cyclic diolefins			
C <sub>5</sub> , cyclopentadiene	1		1
			114



given field can make type analyses of its more important raw materials, intermediates, and products, thereby determining what hydrocarbons are of greatest immediate importance in that field. Then the standard samples can be prepared.

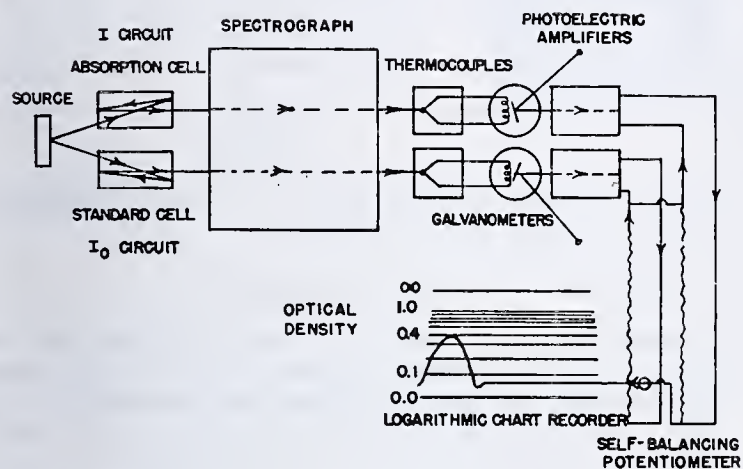


Figure 4. Schematic Diagram of Divided Beam Spectrograph

Regarding the choice and availability of hydrocarbon standards, a number of organizations have contributed as part of the war effort an important fund of information on the hydrocarbons present in aviation gasoline and in materials for synthetic rubber. This includes not only hydrocarbons isolated by analysis as at the National Bureau of Standards and the Pennsylvania State College, but also those prepared by synthesis under the auspices of the American Petroleum Institute—e.g., Ohio State University—and in the laboratories of the National Advisory Committee for Aeronautics, the General Motors Corporation, and several petroleum laboratories. Although some of these hydrocarbons may not be present in commercial streams, it is desirable to have them available as standards to facilitate research on manufacturing methods. Generally it is just as important to have standards representing the undesirable hydrocarbons. For example, in research on aviation gasoline, it is as important to have data on the methylheptanes which have poor antiknock quality as on the desirable trimethylpentanes, in order to obtain accurate analyses on the latter.

With advice from various expert sources, the committee of a newly constituted A.P.I. project (Project 46, Hydrocarbons for Spectrometer Calibration) has drawn up a preliminary list, which it is hoped will include the most useful standards. This is shown in Table II. Some of these hydrocarbons are now available in a relatively pure state, but some will have to be made. All will eventually be purified to a high degree and will be available to those who need them at or near cost. This is obviously a necessary cooperative service to utilize effectively the equipment and methods of spectrometry. It is hoped that contributions will come to that committee (refer to R. P. Anderson, American Petroleum Institute, 50 West 50th St., New York, N. Y.), both in hydrocarbons which

might be available and in suggestions as to important hydrocarbons which should be added to the list.

## PERSONNEL

If it is admitted that the various analytical techniques, new and old, must be used interdependently for greatest effectiveness, it can be seen that an investigator working alone needs to have mastered both fundamental chemistry and fundamental physics and must have developed some degree of skill in instrumentation. When working in a group, the chemist and the physicist can spend more effort in their respective fields; but they must work as an integrated team, so that the procedures worked out will be compatible with the ends in view and with the facilities available. In other words, the analytical procedure should be only sufficiently accurate for the job at hand, should be built around the type of equipment and operators available, and should be changed as circumstances and availability of new facilities dictate. In order to satisfy these requirements it is therefore necessary that close liaison be effected between the analytical staffs and the people who use the analytical results—namely, the manufacturing, development, and research departments.

## INSTRUMENTATION

Among the most important factors contributing to the success of the newer methods have been the skill and the ingenuity of the instrument manufacturers. Without this contribution all the skill displayed by the analyst in the development of techniques would amount to little. As an example, there may be mentioned one recent development which was made by analysts at the Esso Laboratories, but which was made possible by the contributions of the instrument people. This development is the modification of a Gaertner infrared monochromator to permit the recording of infrared spectra directly in terms of optical density. This modification has been of the greatest value in permitting the obtaining of "working" spectra of pure hydrocarbons directly from the instrument with a large saving in time. The modifica-

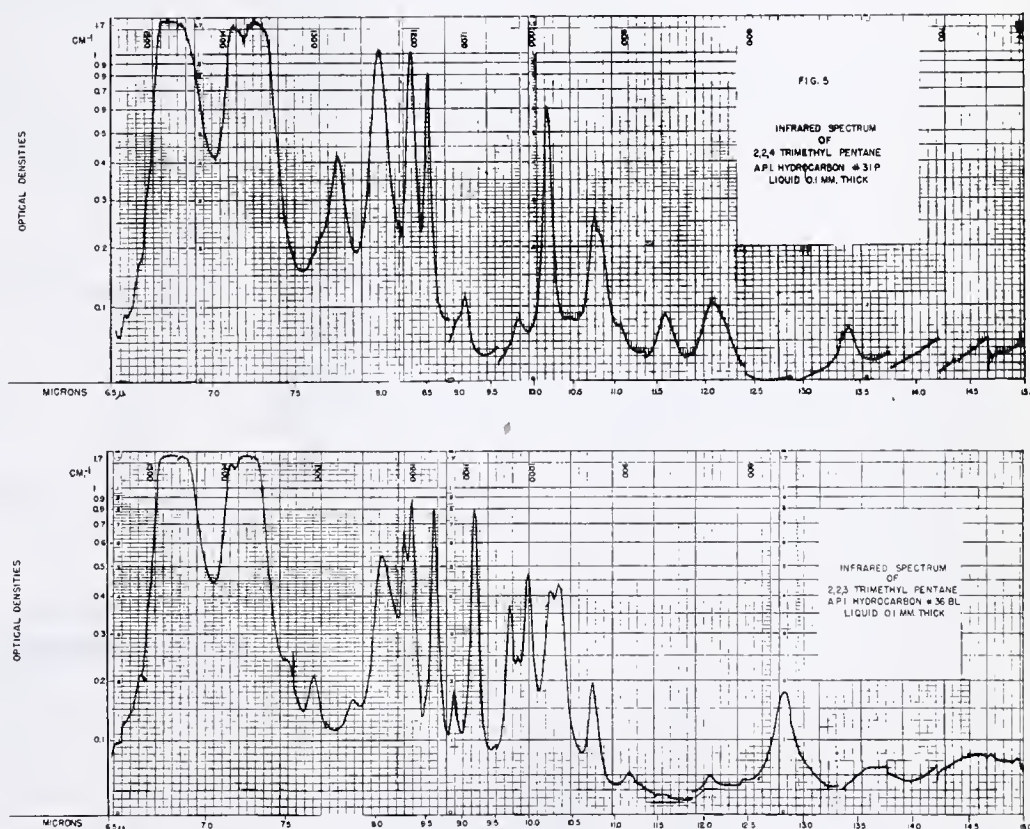


Figure 5. Infrared Spectra

Above, 2,2,4-Trimethylpentane. A.P.I. hydrocarbon 31 P. Liquid 0.1 mm. thick.  
Below, 2,2,3-trimethylpentane. A.P.I. hydrocarbon 36 BL



tions are shown in Figure 4 where the light from the source is split into two beams, one passing through the cell containing the sample and the other through an empty, similar cell, after which both beams enter the monochrometer. The amount of energy from each of these beams is picked up by separate thermocouples, amplified, ratioed with a self-balancing bridge, and the result recorded directly in optical density. Stability and other characteristics essential for good quantitative analysis have been found entirely satisfactory. Figure 5 shows the spectra of 2,2,4-trimethylpentane and of 2,2,3-trimethylpentane as they were taken directly from the recorder.

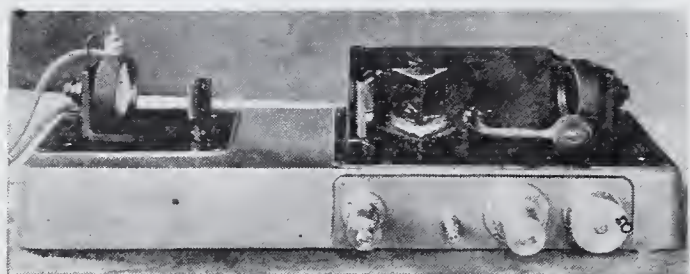


Figure 6. Perkin-Elmer Infrared Spectrometer



Figure 7. Optics of Perkin-Elmer Infrared Spectrometer

The continual introduction of improved instruments is proof that instrument manufacturers recognize their important service to research, development, and manufacturing.

The type of instruments under discussion—for example, infrared and mass spectrographs (6)—requires highly skilled workmanship. Utmost precision is needed in the optical, electrical, and mechanical trains of the apparatus, some of which are custom-built. The price range at the present time usually falls between the limits of \$2000 and \$20,000, depending upon the complexity of construction and the number of units that can be built to standardize design. The Beckman infrared spectrophotometer is already well known as an example of an instrument suitable for routine analyses of gases. An example of a new instrument of moderate price intended for fairly wide service is shown in Figure 6. A partial view of the optics of this Perkin-Elmer infrared spectrometer is shown in Figure 7.

Taking instrumentation with infrared as typical, it is easy to predict that the developments of the future will include automatically recording instruments, monochrometers with higher resolution, more sensitive detecting systems, better amplifiers, more intense light sources, and higher precision machine parts. One would also predict that the improvements to come in the

Table III. Analyses of Synthetic Octane Blend

Constituent	Synthetic Composition	Analy-sis No. 1	Analy-sis No. 2	Analy-sis No. 3	Analy-sis No. 4	Analy-sis No. 5
2,2,4-Trimethylpentane	27.8	27.0	28.0	28.2	27.4	27.9
2,5-Dimethylhexane	1.7	0.0	2.3	1.8	1.4	2.3
2,4-Dimethylhexane	1.6	2.9	0.2	0.0	2.6	2.1
2,2,3-Trimethylpentane	30.7	32.2	32.1	32.4	31.4	31.0
2,3,4-Trimethylpentane	29.5	30.6	31.4	30.6	30.4	30.1
2,3,3-Trimethylpentane	6.8	6.4	6.0	7.0	5.9	6.6
2,3-Dimethylhexane	1.9	0.9	0.0	0.0	0.9	0.0

other of the newer instruments will be carried to a similar degree where such improvements are needed.

#### TYPICAL APPLICATION

Applications of infrared to hydrocarbon analyses are adequately covered by Brattain and co-workers (1). This subject can be taken as a case in point for further amplification, being applied along with distillation to the analysis of the  $C_8$  paraffins, as an example of what the petroleum technologist in cooperation with the instrument designer can do. One important application of infrared spectrometry, when it is combined with suitable distillation, is the analysis for the individual paraffins in a mixture such as a commercial "isooctane" fraction. Distillation is an integral part of this program, to obtain cuts having not more than 8 components and to make separations where spectral differences of the hydrocarbons are not so pronounced as might be wished. In Table III there are presented the infrared analyses of one synthetic mixture containing 7 of the isomeric octanes, to show that both accuracy and reproducibility are well within the limits required for most applications.

Previously, analyses of such mixtures as paraffinic alkylates have been done in an empirical way on the basis of boiling points in an "analytical distillation". In general, this has required a

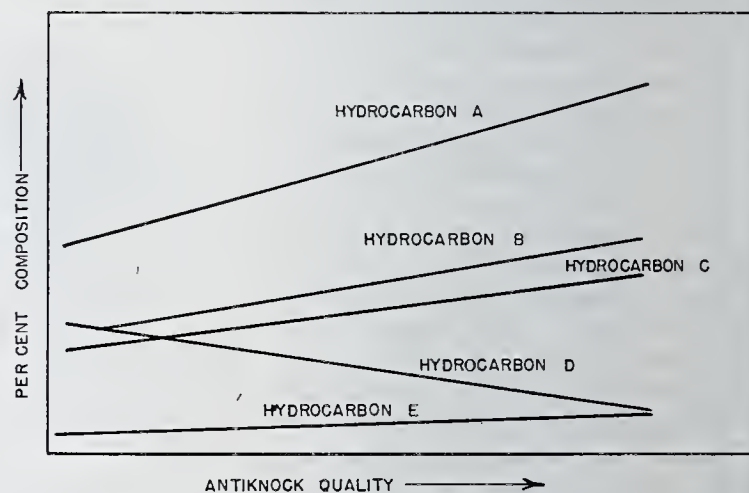


Figure 8. Relationship between Composition and Antiknock Quality

Table IV. Infrared Analysis of Contiguous Cuts Distilled from Experimental Fuel

Boiling Range	94-99° C.	99° C.	99-100° C.	100-109° C.	109-112° C.	113-114° C.	114° C.
2,3-Dimethylpentane	17	..	..	..	..	..	..
2,2,4-Trimethylpentane	83	98	91	35	4	0	0
2,5-Dimethylhexane	..	1	6	35	30	14	8
2,4-Dimethylhexane	..	1	2	15	16	14	7
2,2,3-Trimethylpentane	..	0	1	9	12	8	6
2,3,4-Trimethylpentane	..	0	0	5	30	45	51
2,3,3-Trimethylpentane	..	..	..	0	8	19	26
2,3-Dimethylhexane	..	..	..	1	0	0	2



Table V. Analyses

Ultraviolet Absorption	Routine Infrared	Research Infrared
Benzene	Isobutane- <i>n</i> -butane	Individual C <sub>6</sub> paraffins
Toluene	Isopentane- <i>n</i> -pentane	Individual C <sub>7</sub> paraffins
C <sub>8</sub> aromatics	C <sub>3</sub> -C <sub>4</sub> -C <sub>5</sub> paraffins	Individual C <sub>8</sub> paraffins
C <sub>9</sub> aromatics	Isobutylene purity	Cuts of alkylate
Butadiene	Isobutylene	Cuts of hydrocodimer
	Methyl chloride	Cyclopentane
	Isobutane	C <sub>8</sub> saturates
Ultraviolet Emission		Amylenes
Potassium	Mass Spectrograph	Isohexenes
Barium		Benzene
Lead	Exhaust gas	Toluene
	C <sub>4</sub> cut	C <sub>8</sub> aromatics
	Paraffin gases	C <sub>9</sub> aromatics
	Flue gas	
Raman		
C <sub>8</sub> aromatics		
C <sub>9</sub> aromatics		
Simple paraffins		

large background of experience on similar products, for experience has shown that distillation plateaus in petroleum distillates represent more than one component when complex mixtures are distilled.

As an example, Table IV gives the infrared analyses of blended cuts prepared by Fenske (2) from the distillation of an experimental fuel in a still of 1-barrel capacity, with a fractionating column having 100 equivalent plates. Considering 2,2,3-trimethylpentane (b.p. 109.8° C.) it will be noted that this compound begins to show in the cut taken off at 99-100° C., increases to a maximum in the cut taken off at 109-112° C., and is still present to an appreciable extent in the cut taken off at 114° C. At the time the distillation was made the infrared technique was not available; with the aid of refractive indices and other physical constants, it was possible to translate the distillation data to accurate analyses in terms of the major components. However, his interpretation required much more experience and time than were subsequently needed when the infrared technique was applied.

A large number of aviation fuel-blending agents have been analyzed by infrared and the data have been found useful in a practical way for guiding research and process control. Figure 3 shows empirically the effect of change in composition of a blending agent on antiknock quality, the change in composition being due to changes in operating conditions.

Table VI. Savings by Optical Methods

Method	Time Hours	Time by Other Methods Hours
Benzene and toluene	Ultraviolet	0.75
C <sub>8</sub> aromatics	Ultraviolet	1
Butadiene	Ultraviolet	0.25
Potassium	Ultraviolet	1
Barium	Ultraviolet	0.75
Sodium	Ultraviolet	1
Lead	Ultraviolet	1
Total C <sub>4</sub> cut	Infrared	1
Isobutane and <i>n</i> -butane	Infrared	0.25
Isopentane and <i>n</i> -pentane	Infrared	0.167
C <sub>3</sub> , C <sub>4</sub> , and C <sub>5</sub> paraffins	Infrared	0.75
C <sub>6</sub> paraffins	Infrared	6
C <sub>7</sub> paraffins	Infrared	6
C <sub>8</sub> paraffins	Infrared	2
C <sub>9</sub> olefins	Infrared	3

Regarding more general applications of spectrometry, Table V lists a number of analyses found possible to perform by spectrographic means. This list by no means exhausts all the possibilities. In particular, much wider application of the emission spectrograph should prove a valuable asset in analytical work.

As to the savings which are possible by optical methods, an attempt has been made to present a rough estimate in Table VI. In almost all the cases shown there is an appreciable saving in time over conventional methods. In some cases, the advantage of the spectrographic method is more than 200 hours. In prac-

tice, these older time-consuming methods would rarely, if ever, be used. The estimates given in parentheses are not so firm as the others, but they are reasonable for methods based upon fractional distillation at very high reflux ratios.

## SUMMARY

Much progress has been made in applying the newer techniques of physics to hydrocarbon analysis, and greater advances are to be expected. However, no one method is self-sufficient, and its usefulness is dependent largely on judicious combination with other methods. It might be expected that the marked decrease in time required to complete an analysis should result in a decrease in operating costs of an analytical laboratory. It may happen, however, that when the utility of such analyses becomes apparent, much more analytical work is undertaken. In such a case the total cost may go up; but this may be fully justified by a marked gain in speed of research, control of operations, and improvement of product quality.

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## Standards for Analytical Filter Papers

Methods have been developed at the National Bureau of Standards, Washington, D. C., for testing filter papers and applying the methods to the establishment of standards of quality. These include rate of flow of water, retention of fine precipitates, and bursting strength of wet paper, and an improved method for determination of ash content. Other tests considered by the bureau as desirable for a thorough evaluation of filter papers are thickness, weight per unit area, alpha-cellulose, copper number, and pH.

The bureau has completed testing the various types and grades of analytical filter papers made in England, Sweden, and the United States, and found them all to be of good and in most respects of equal quality.

**CORRECTION.** In the article on "Sulfuric Acid Extraction in Hydrocarbon Type Analysis" [*IND. ENG. CHEM., ANAL. ED.*, **16**, 558 (1944)], two errors appeared. The estimated accuracy of the volume per cent of saturates should be  $\pm 2\%$  instead of  $\pm 0.2\%$  as printed. In Table III, column 2, the last blend number should be 7 instead of 6 as printed.

CLYVE C. ALLEN



# Spectrochemical Analysis with the Air-Acetylene Flame

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IN THE spectrochemical analysis of biological and related materials certain elements may be encountered occasionally at levels of concentration that do not require methods of maximum sensitivity. In such cases the prime requisites are reproducibility and accuracy of results and convenience of procedure, qualities which are more easily met by the air-acetylene flame method than by any other form of excitation. In spite of this and other advantages, spectrographers in this country have made little use of the flame method, and therefore the authors' experience in the adaptation and application of technical procedures is given in this article. Much of the material is not original, but the information is widely scattered in the literature and since many scientific journals are not readily available at the present time, its presentation should be useful.

## PRINCIPLE

With the air-acetylene flame it is possible to detect 34 elements (5), among which are those of greatest interest from a biological standpoint. The elements that can be detected are indicated within the blocked-off portions of Table I. The approximate molar sensitivities of detection are indicated in footnotes. The flame is used in the analysis of solutions which are carried as fine mists in the air supplied to the acetylene. The resulting light is then analyzed (photographed) by means of an appropriate quartz spectrograph. The photographed lines are measured with a suitable densitometer, the densities of the lines obtained under rigidly controlled conditions of exposure and photography being proportional to the amount of element which produced them.

## EQUIPMENT

Figure 1 illustrates the atomizer and burner, while Figure 2 shows the equipment in place for use. In details of construction this equipment follows Lundegårdh's (6, 7) latest design as described by McClelland and Whalley (8). Although the general construction is evident from the illustration, a number of measurements and details have been omitted to make the drawing simpler, and these are given below. Ready-built atomizers and burners may be imported at an approximate cost of \$900, but the equipment illustrated was built for about \$200, the most costly item being the fabrication of the platinum-iridium components. The cost may be further reduced by using less expensive and less resistant alloys for the metal parts, but these may have to be replaced frequently.

Air at 2.8 kg. per sq. cm. (40 pounds per square inch) is taken from a compressor and passes through the brass tube, A. The air in passing through the small hole in C (platinum-iridium jet 0.4 cm. in outside diameter, 0.2 cm. in inside diameter except 0.04-cm. opening at top) and the platinum-iridium disk, F (0.1-cm. hole widening to a 45° angle) sucks up the fluid which has entered the ebonite lower portion of nozzle D (1.2 cm. in diameter) and carries it as a spray through the opening (0.3 cm.) in

the adjustable ebonite upper portion of nozzle E. (The brass tube, A, to which C and D are fixed, is held in place in the glass 19/38" male joint by means of the lock nut, B, and a rubber washer.) The larger droplets of the spray issuing from E are returned to the main portion of the solution when they strike the walls of the glass vessel, while the air carrying the fine mist is led to the burner through G. Opening G must be large enough, so that liquid flowing down the walls is not forced through it; if this should occur, the liquid would fill the narrower opening in which this outlet ends, thus producing a pulsating air stream through the burner. The opening in D should be made low enough so that the atomizer can handle 2 cc. of sample if required to do so. The atomizer illustrated, when set in a vertical position, can handle 5 to 20 ml. of solution, but when set at an angle it works well with only 2 ml.

The air compressor is operated at pressures between 6.7 and 8.1 kg. (95 and 115 pounds) by means of a Cutler-Hammer, Style D, regulator switch and is provided with an outlet carrying a 3.5-kg. (50-pound) maximum adjustable pressure regulator.

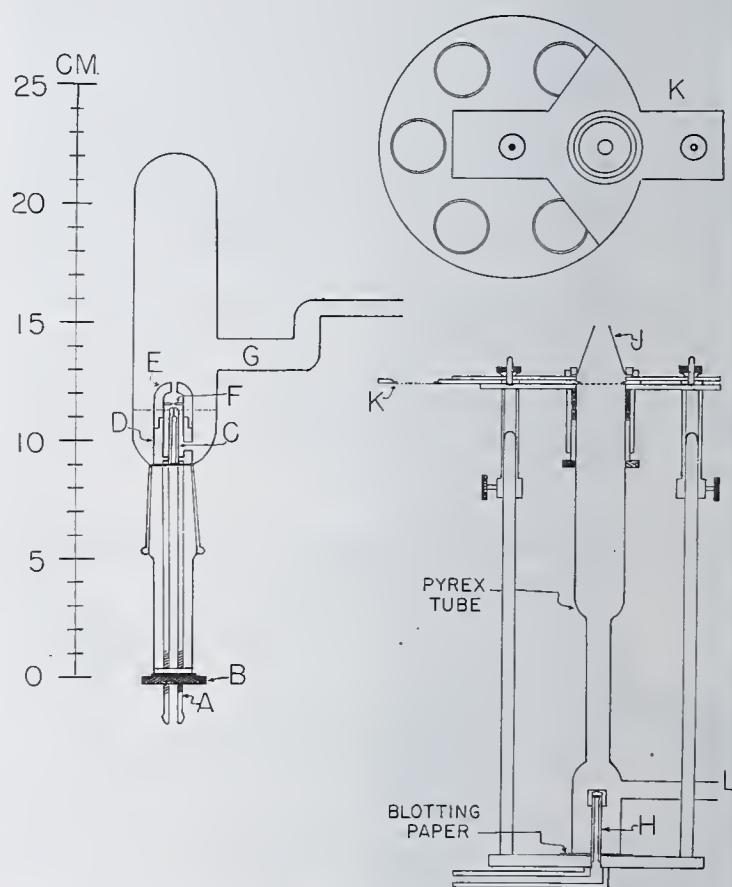


Figure 1. Details of Construction of Lundegårdh Atomizer and Burner

Table I. Elements Susceptible of Flame Analysis

Shell	I	II	III	IV	V	VI	VII	VIII	O		
1	1 H	.....	.....	....	...	....	.....	....	2 He		
2	3 Li <sup>a</sup>	4 Be	5 B	6 C	7 N	8 O	9 F	....	10 Ne		
3	11 Na <sup>a</sup>	12 Mg <sup>b</sup>	13 Al	14 Si	15 P	16 S	17 Cl	....	18 A		
4	19 K <sup>b</sup>	20 Ca <sup>a</sup>	21 Sc <sup>d</sup>	22 Ti	23 V	24 Cr <sup>b</sup>	25 Mn <sup>a</sup>	26 Fe <sup>b</sup>	27 Co <sup>b</sup>	28 Ni <sup>b</sup>	36 Kr
5	29 Cu <sup>a</sup>	20 Zn <sup>c</sup>	31 Ga <sup>d</sup>	32 Ce	33 As	34 Se	35 Br	....	....	....	...
6	37 Rb <sup>a</sup>	38 Sr <sup>a</sup>	39 Y <sup>d</sup>	40 Zr	41 Cb	42 Mo	43 ...	44 Ru <sup>a</sup>	45 Rh <sup>d</sup>	46 Pd <sup>b</sup>	54 Xe
7	47 Ag <sup>a</sup>	48 Cd <sup>c</sup>	49 In <sup>d</sup>	50 Sn	51 Sb	52 Te	53 I	....	....	....	...
8	55 Cs <sup>b</sup>	56 Ba <sup>b</sup>	57-71	72 Hf	73 Ta	74 W	75 Re	76 Os	77 Ir	78 Pt	86 Rn
			Rare earths <sup>e</sup>								
	79 Au <sup>a</sup>	80 Hg <sup>c</sup>	81 Tl <sup>a</sup>	82 Pb <sup>b</sup>	83 Bi	84 Po	85...	....	....	....	...
9	87...	88 Ra	89 Ac	90 Th	91 Pa	92 U	93...	94...	....	....	...

<sup>a</sup> 0.0001 molar. <sup>b</sup> 0.001-0.0001 molar. <sup>c</sup> 0.001 molar. <sup>d</sup> Undetermined. <sup>e</sup> Rare earths, Dy, Gd, La, Nd, and Pr, all undetermined.



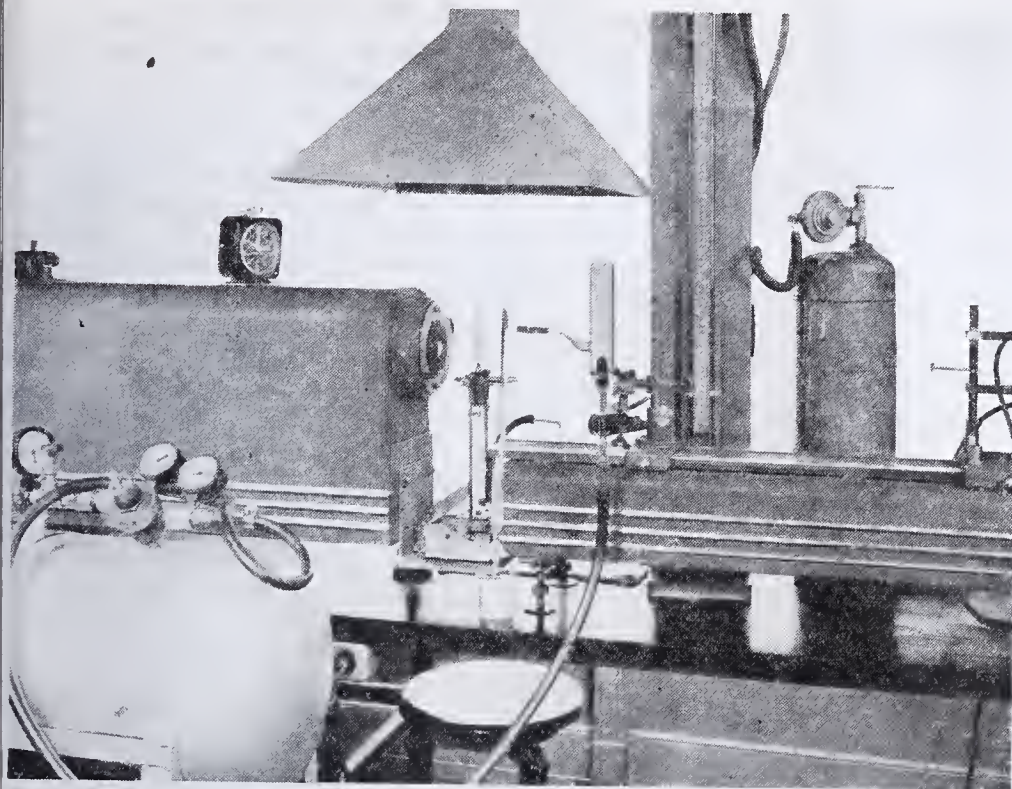


Figure 2. Burner Equipment Set Up for Use

In the moderate range of pressures at which the compressor operates, the regulator delivers air at  $2.8 \pm 0.035$  kg. per sq. cm. ( $40 \pm 0.5$  pounds per square inch) pressure.

The mist issuing from *G* is led to the burner through *L* and is mixed with acetylene in the narrow portion of the Pyrex tube. Acetylene at 25-cm. water gage enters the burner through *H* (platinum-iridium jet 0.4 cm. in outside diameter, 0.2 cm. in inside diameter, with screw cap of same material holding in place a platinum-iridium disk 0.3 cm. in diameter with a centered hole 0.05 cm. in diameter). The air-acetylene mixture issues through a platinum wire screen (45-gage, 0.002-cm., 0.008-inch, wire) carried in the rotatable disk, *K*, and finally passes through the platinum-iridium cone, *J*.

Acetylene is obtained from a Prest-O-lite Type B (40 cubic feet) cylinder and is maintained at  $25 \pm 0.2$  cm. water gage by means of a Type OO (10-pound) Prest-O-lite regulator and a second needle valve between the regulator and the water manometer. Before entering the burner the acetylene is passed through a water bubbler which keeps the gas moist and prevents clogging of the orifice in *H* by material which otherwise would deposit, because of drying of the mist (8). The bubbler also serves to remove minute particles of cylinder-packing material which would clog the small orifice. It is good practice, before inserting a full Prest-O-lite cylinder, to blow off (in the open air) a sufficient amount of acetylene to make certain that acetone spray is not carried along with the acetylene.

The burner is so placed that the cone of the flame is in the optical axis and in the case of a Bausch & Lomb medium quartz spectrograph, it is set 5.5 to 6.0 cm. from the slit. Its height is so adjusted that the tip of the blue inner cone of the flame is about 10 mm. below the opening of the slit of the spectrograph. In this position maximum intensity is obtained (5) and the amount of light entering the slit is further increased by reflection from a plane chrome-plated mirror set immediately behind the flame (not shown in Figure 1). A smaller spectrograph than the one indicated can be employed, but its dispersion must be sufficient to separate cleanly the manganese triplet at 4031 Å. from the potassium doublet at 4044/7 Å. In the particular work described the standard period of exposure was 2 minutes. Eastman No. 33 plates were used for the determination of all lines from Mg 2852 Å. to Sr 4607.3 Å., and Panchromatic plates for lines at longer wave lengths, particularly for Na 5890/5.9 Å. and Li 6707.9 Å.

SENSITIVITIES

In practice the sensitivity and the reproducibility obtainable depend on a strict standardization of the details of operation. Each atomizer and burner operates most efficiently and yields

maximum sensitivity at air and acetylene pressures which are unique, dependent upon the size of the orifices of the spray and acetylene jet. Lundegårdh worked at air pressures varying from 30 to 180 pounds per square inch and 35-cm. water gage acetylene (5); McClelland and Whalley employed air at 100 pounds per square inch, 40-cm. acetylene (8); Ells (2) air at 30 pounds per square inch and acetylene at 22 cm.; while Griggs and co-workers (4) used air at 37.5 pounds per square inch and acetylene at 26 cm. The authors used air at 40 pounds per square inch and acetylene at 25 cm. These conditions were chosen as the most suitable for the simultaneous analysis of certain concentrations of magnesium, copper, sodium, iron, manganese, potassium, calcium, and strontium when all of these cations or various combinations of them were present in the samples. Whenever ultimate sensitivity is required, however, each element presents a problem in itself and the best conditions for its detection must be determined beforehand.

The variations in the line intensities of certain elements, due to varying air pressures at a fixed acetylene pressure of 25 cm., are graphically illustrated in Figure 3. These relative intensities were obtained by means of an intensity calibration standard placed on the plate with a step sector. The density-intensity plot of the plate calibration was made as described by Pierce and Nachtrieb (12) for use in correcting for background. Figure 3 demonstrates that with an acetylene pressure of 25 cm. the air pressures which are satisfactory for the maximum sensitivity of detection of sodium and manganese, among others, are not suit-

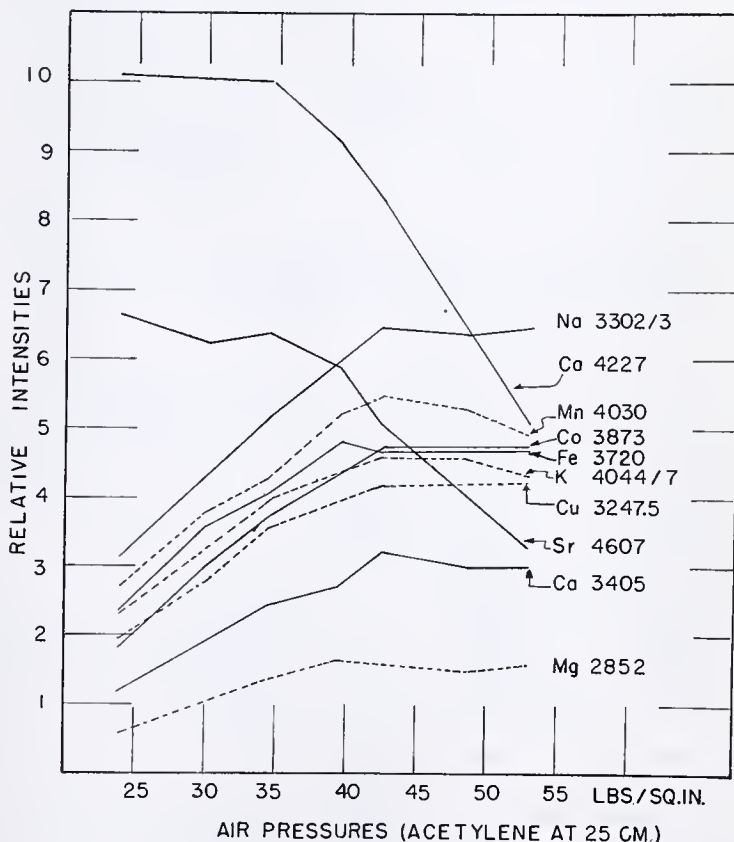


Figure 3. Variations in Line Intensities Obtained with Variations in Air Pressure



## PHOTOMETRY AND REPRODUCIBILITY

Table II. Comparison of Sensitivities of Detection by Flame and Arc Excitation

Element	Line Å.	Sensi- tivity Mg./l.	Element Passing through Burner in 2- Min. Exposure	Element on Arc A.C. and D.C.
			Mg.	Mg.
Ag	3280.7	5	$1.5 \times 10^{-3}$	$1 \times 10^{-5}$
Au	2676	20	$6.0 \times 10^{-3}$	.....
Ba	5535.5	100	$3 \times 10^{-2}$	.....
Ca	4226.7	0.4	$1.2 \times 10^{-4}$	$1 \times 10^{-4} - 2 \times 10^{-5}$
Cd	3261.1	200	$6 \times 10^{-2}$	$2 \times 10^{-4} - 1.7 \times 10^{-5}$
Co	3526.9	1	$3 \times 10^{-4}$	$1.1 \times 10^{-4}$
Cr	3578.7	0.5	$1.5 \times 10^{-4}$	$1 \times 10^{-5}$
Cs	4553.3	50	$1.5 \times 10^{-2}$	.....
Cu	3247.5	0.5	$1.5 \times 10^{-4}$	$1.7 \times 10^{-4} - 5 \times 10^{-5}$
Fe	3849.9 <sup>a</sup>	5.0	$1.5 \times 10^{-3}$	$6 \times 10^{-5} - 5 \times 10^{-6}$
Hg	2536.5	200	$6 \times 10^{-2}$	$2 \times 10^{-4}$
K	4044.2/7.2	8	$2.4 \times 10^{-3}$	.....
Li	6707.9	0.1	$3 \times 10^{-5}$	$1.4 \times 10^{-5}$
Mg	2852.1	5.0	$1.5 \times 10^{-3}$	$6 \times 10^{-5} - 1 \times 10^{-5}$
Mn	4030.8 <sup>b</sup>	0.3	$9 \times 10^{-5}$	$1.3 \times 10^{-3} - 1 \times 10^{-6}$
Na	5890/5.9	0.2	$6 \times 10^{-5}$	.....
	3302.3/2.9	11.5	$3.5 \times 10^{-2}$	$2 \times 10^{-3}$
Ni	3414.8	10	$3 \times 10^{-3}$	$2 \times 10^{-4} - 1.5 \times 10^{-5}$
Pb	4057.8	100	$3 \times 10^{-2}$	$1 \times 10^{-4} - 8 \times 10^{-7}$
Pd	3634.7	20	$6 \times 10^{-3}$	.....
Rb	4201.8	10	$3 \times 10^{-3}$	.....
Ru	3725.9/8.0	10	$3 \times 10^{-3}$	.....
Sr	4607.3	0.2	$6 \times 10^{-5}$	$6 \times 10^{-5} - 5 \times 10^{-6}$
Tl	3775.7	0.4	$1.2 \times 10^{-4}$	$2 \times 10^{-5}$
Zn	3072.1	3000	9	$2 \times 10^{-3} - 3 \times 10^{-4}$

<sup>a</sup> 3719.9 is somewhat more sensitive.<sup>b</sup> This is really a triplet.

able for the most sensitive detection of calcium and strontium. In the first case maximum sensitivity is obtained at 42.5+ pounds' air pressure, and in the second case, between 25 and 30 pounds' air pressure. Therefore when calcium and strontium must be determined simultaneously with other elements, the conditions chosen must be the most favorable for the detection of the cation present in the smallest amount. When the above test is repeated at a lower acetylene pressure (22 cm.) the shapes of the intensity variation curves are essentially the same, but occur at lower levels. Some increase in the intensity levels is obtained at higher acetylene pressures, but the gain for all practical purposes is not sufficiently great to warrant the increased use of acetylene.

Lundegårdh (5) has determined the ultimate sensitivities of detection of most of the elements detectable by the air-acetylene flame. These are given on a concentration (milligrams per liter) basis in Table II, where the most suitable analytical lines are also indicated. Since the ultimate sensitivity depends on the quantity of sample passed through the flame, a calculation based on this quantity is included in the table. The sensitivities are on the conservative side, since they were based on the use of equipment by means of which approximately 0.300 gram of solution passed through the burner as a mist in 2 minutes. This weight was obtained by blowing air at 40 pounds per square inch through a weighed sample of water and determining the difference in weight at the end of 20 minutes. Duplicate runs for water and for a sample simulating ashed blood plasma showed good agreement, but since the burner itself was not in the air stream, the amount calculated as consumed may be somewhat high, particularly since the removal of the larger particles of mist in the burner itself was not considered in obtaining the difference in weights. Lundegårdh (5) has described an atomizer in which only about 0.075 gram is consumed in 2 minutes; with his equipment (including a smaller spectrograph) the amounts detected were one fourth those given in the table. When the ranges of sensitivities calculated from the actual amounts of samples passing through the flame are compared with the ranges of sensitivities reported for the high-voltage alternating current arc by Owens in the analysis of various materials (11), and by Cholak and Story (1) for the analysis of biological material by the direct current arc, it becomes obvious that the sensitivities are higher than is apparent from consideration of the milligram per liter value. If suitable concentration of the sample can be accomplished, the analytical range for many elements is only slightly inferior to that obtained with the stronger excitation methods.

Investigators who have used the air-acetylene flame usually make use of the photometric procedure advocated by Lundegårdh (5), which is based on the concept that the air-acetylene flame is so stable and reproducible that internal standard lines need not be used. As a consequence the background, which is produced mainly by the flame, serves as the internal standard, and accordingly the ratios obtained by dividing the galvanometer reading for the line by that for the neighboring background (L/H) varies with the concentration, in the flame, of the element producing the line. An alternative method, called the L-H method, is also used occasionally. In this method the differences in the galvanometer readings between the lines and the backgrounds are used in place of the ratios. The standardization curves resulting from these data are illustrated for iron in Figure 4. Both curves show appreciable curvature at the higher levels of concentration, and consequently a decrease in the accuracy of analysis in this region is implied.

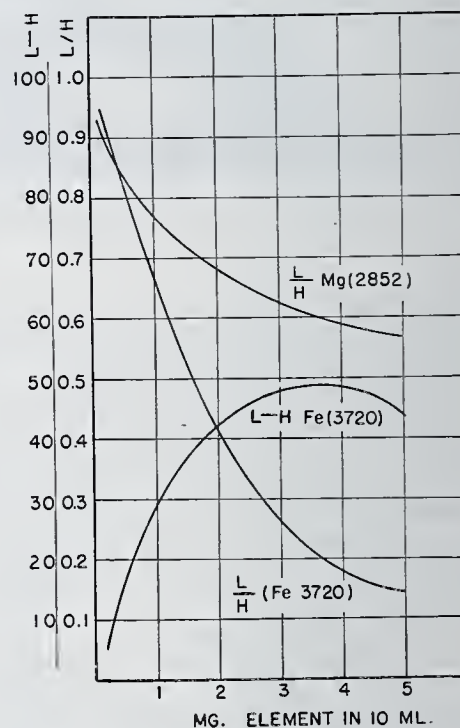


Figure 4. Typical Analysis Curves Obtained with L/H or L-H Method of Photometry

In order to correct for variations in the sensitivity of individual plates, as well as for slight variations in the excitation conditions, Lundegårdh derives a master curve by averaging the results for the standard solutions taken on numerous plates. Samples to be analyzed are next placed on the same plate with a series of standard solutions. The L/H ratios of the samples are then corrected by applying a factor representing the mean ratios between the L/H values of the standard solutions on the particular plate and the corresponding L/H values of the master curve.

This procedure has been used successfully, but it appears to have a number of disadvantages. A more convenient calibration curve, particularly one with a linear response over the entire analytical range, would increase the analytical accuracy at higher concentrations and would reduce considerably the frequency with which an analysis must be repeated after dilution of a sample to bring it into the most favorable portion of the L/H curve (5). This apparently can be accomplished merely by altering the manner in which the data are plotted. If the L/H ratios are plotted on a logarithmic scale, a more linear calibration results, as can be seen from Figure 5. In the particular case



Table III. Analytical Lines, Internal Standards, and Analytical Ranges

Element	Wave Length	Internal Standard	Analytical Range, Mg./10 Ml.
Magnesium	2852	Co 3405	0.05-5.0
Copper	3247.5	Co 3405	0.04-2.5
Sodium	3302/3	Co 3405	0.30-25.0
Iron	3719.9	Co 3873	0.05-5.0
Manganese	4030	Co 3873	0.005-0.30
Potassium	4044/7	Co 3873	0.06-4.0
Calcium	4227	Co 3873	0.005-0.20
Strontium	4607	Co 3873	0.004-0.30

illustrated, the 5-mg. value is slightly high, causing a slight deviation from linearity for values above 3 mg., for the reason given below.

The method of correcting for variations in individual plate sensitivity in the L/H method is inconvenient, time-consuming, and uneconomical. Moreover, it does not correct truly for variations in excitation conditions arising from differences in the quantity of mist produced in atomizing samples of varying composition and viscosity. Variation in the quantity of mist in the flame will affect the intensity of the test line to a much greater extent than it affects the background, since the latter is due mainly to the flame. Neither method (L/H or L-H) corrects properly for the effect of deep background which arises from the sample itself. This is most clearly demonstrated in the L-H curve of Figure 4, where the background subtractions are too high, since the L-H value for 5 mg. of iron is lower than that obtained with 2.5 mg. of iron. When using the L/H method under the same conditions, the L/H values will be high and they will tend to flatten the curves at the higher concentrations, as in Figure 4, or cause them to deviate from the linear relationship, as indicated in Figure 5.

Reproducible results therefore can be obtained with the L/H or L-H method only by the strict standardization of all the possible variants. In spite of the fact that in the flame as compared to the arc there is little interionic action to affect line intensities, the presence of moderate quantities of certain cations (magnesium, sodium, potassium, iron, and aluminum, among others) will produce marked changes in the background. As a result it is not possible with the L/H or L-H method to derive a single analytical curve which can be used with equal accuracy for the analysis of materials showing large differences in matrix composition.

All the above-mentioned disadvantages, however, disappear if internal standard lines are used in place of background and if the plate calibration and background correction of modern photometry are employed.

The method used by the authors is the conventional one, in which the intensity ratio (*I* line/*I* standard) is obtained from the plate intensity calibration pattern made for each batch of plates, the intensity of each line being corrected for the background in which it lies by the method of Pierce and Nachtrieb (12, 13). Plate intensity calibration marks are obtained with a rotating step sector (6 steps, factor of 2) and a low amperage arc (2.5 amperes) across 7-mm. diameter brass rods. Eastman No. 33 plates are used for the analysis of all lines from 2852 to 4607 Å. and because of the variation of gamma with wave length separate plate calibrations are obtained for the 2850 to 3600 Å. and for the 3600 to 4607 Å. regions. The slopes of the H and D curves taken at a number of wave lengths in each region show such slight differences that no appreciable error is introduced by the use of a single standard line

for each region. The slopes of the group of H and D curves for the 3600 to 4600 Å. region, however, are somewhat greater than those for the shorter wave-length region and therefore a separate calibration must be obtained for each region.

Concentration calibrations were obtained for eight elements by diluting serially a stock solution containing all the elements of interest in addition to 10% hydrochloric acid and 5 mg. of cobalt per 10 ml. of solution. The diluent consisted of a 10% hydrochloric acid solution of cobalt chloride, 5 mg. of cobalt per 10 ml. The cobalt line at 3405 Å. was used as the standard for all lines between 2850 and 3600 Å., while the cobalt line at 3873 Å. was employed for analysis in the region between 3600 and 4607 Å. Two-minute exposures, air pressures of 40 ± 0.5 pounds per square inch, and acetylene pressures of 25 cm. were employed. These conditions were chosen for the reasons given under "Sensitivities" and also because at the adopted conditions the intensity ratios showed practically no variation due to small changes in the excitation conditions.

The elements for which analytical curves have been derived, the internal standard lines used, and the analytical ranges covered (expressed as milligrams per 10 ml. of solution) are given in Table III. The lower concentration values in many cases can be reached only by doubling or tripling the exposure period. Tests on standard solutions have shown that the intensity ratios given by the increased exposures fit the values obtained by extrapolating calibration curves derived at higher concentrations and with 2-minute exposures. The characteristic analytical curve obtained when log intensity ratio is plotted against log concentration is illustrated in Figure 6, the data being taken

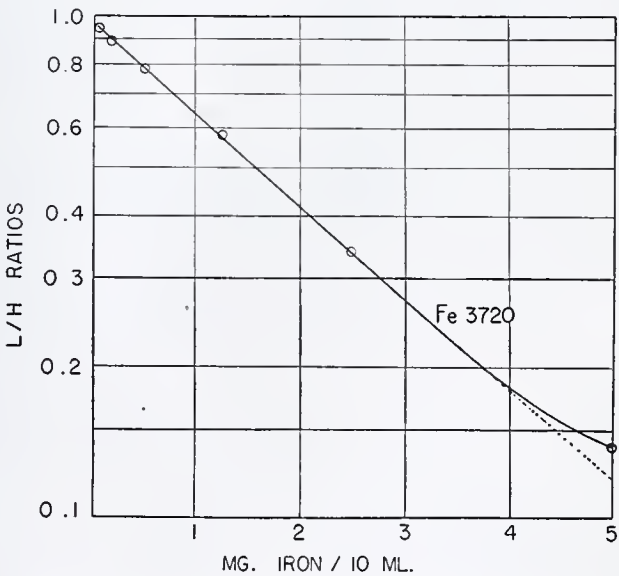


Figure 5. Analytical Curve Obtained When L/H Ratios Are Plotted Logarithmically

Table IV. Reproducibility of Results Obtained in Repeated Analyses of Same Sample

Element	Amounts Added Mg.	Expected Ratio from Standard Curve	Mean Ratio Obtained	Std. Dev.	Standard Deviation % or Coefficient of Variability	Values from Calibration Curve Mg.	Spread Calculated as Due to Standard Deviation %
Individual Spectra							
Magnesium	1.50	0.62	0.61	±0.053	±8.7	1.49	1.35 -1.62
Copper	0.60	1.40	1.46	±0.067	±4.6	0.65	0.62 -0.68
Sodium	7.50	2.25	2.30	±0.068	±2.95	7.60	7.4 -7.8
Iron	1.50	1.07	1.07	±0.033	±3.1	1.50	1.46 -1.55
Manganese	0.09	1.23	1.21	±0.041	±3.4	0.089	0.086-0.092
Potassium	1.2	0.98	1.04	±0.033	±3.2	1.25	1.21 -1.29
Calcium	0.12	2.20	2.08	±0.11	±5.3	0.112	0.106-0.118
Strontium	0.09	1.35	1.35	±0.042	±3.1	0.09	0.088-0.092
Duplicate Spectra							
Magnesium	1.50	0.62	0.61	±0.046	±7.6	1.49	1.38 -1.60
Copper	0.60	1.40	1.45	±0.057	±3.9	0.64	0.61 -0.66
Sodium	7.50	2.25	2.30	±0.041	±1.8	7.60	7.45 -7.75
Iron	1.50	1.07	1.07	±0.025	±2.3	1.50	1.47 -1.53
Manganese	0.09	1.23	1.21	±0.025	±2.1	0.089	0.087-0.090
Potassium	1.2	0.98	1.03	±0.016	±1.55	1.25	1.23 -1.27
Calcium	0.12	2.20	2.08	±0.075	±3.6	0.112	0.108-0.116
Strontium	0.09	1.35	1.34	±0.024	±1.8	0.09	0.088-0.091



## APPLICATIONS

Table V. Effect of Size of Sample on Analytical Reproducibility

Ml. of Original Sample in Atomizer	Ca	Mg. of Element Mg	per Liter of Tap Water Na	K	Fe	Sr
1	31.0	..	..	...	..	...
5	29.0	..	..	..	..	...
100	..	5.0	..	1.6	..	0.13
200	..	5.25	9.5	1.5	..	0.135
500	..	4.9	10.0	1.6	0.11	0.126

Table VI. Effect of Method of Sample Preparation on Cation Recovery

Technique	Mg	Grams per Liter of Urine Na	K	Ca
Dry ashing	0.10	3.55	2.0	0.029
Sulfuric acid-nitric acid digestion	0.115	4.00	2.20	0.037
Nitric acid-hydrogen peroxide digestion	0.110	3.90	2.20	0.037
Untreated	0.10	3.90	2.10	0.035

from the same plate used for Figures 4 and 5. The slopes of all the concentration calibration lines except that for magnesium show only slight variation from that of the iron analytical line, the differences being due to the reversibility shown by some of the lines (12). The greater steepness of the magnesium-analytical line cannot be attributed solely to the factor of reversibility, but regardless of the real reason for this phenomenon it is evident that the magnesium plot in Figure 6 is superior for analytical purposes to the very shallow L/H curve of Figure 4.

Also shown in Figure 6 is an analytical curve for iron based on intensity values alone, corrected for the background intensity as employed in the intensity ratio method. The straight line obtained indicates that this method more nearly corrects for background than does the analogous L-H method. Obviously, such a calibration can be used for analytical purposes, but its use has the disadvantage of requiring the same method of correcting for variations in individual plate sensitivity that is used in the L/H or L-H method.

In Table IV are listed data which illustrate the reproducibility possible with the use of the log intensity ratio-log concentration method of plotting the photometric values. These data were calculated from twenty spectra of the same solution taken on a single plate. The stock solution used in preparing the known concentrations was similar to that used in deriving the standard analytical curves, but was freshly made up to replace the depleted original stock solution. In some instances this accounts for the small differences between the mean values of the twenty spectra and the expected ratio obtained from the standard curve. The statistical analysis was made on the ratio values obtained, but corresponding concentration values are also given for these data. By way of comparison, it is interesting to note Mitchell's study of 15 spectra of a 0.000125 *M* manganese chloride solution (9), in which he obtained a standard deviation of  $\pm 6.41\%$  in the L/H ratios of the single spectra and  $\pm 4.5\%$  when duplicates were used. Lundegårdh (5) reports that in general the error of determination never exceeds 5 to 7%, and in dealing with the elements calcium and manganese, the errors are not more than 1 to 2%.

The errors of analysis indicated in Table IV may have been increased by the use of a single calibration pattern for plates from the same production batch. In all probability the portion of the error due to the photometric procedure can be reduced by placing an intensity calibration pattern on each plate.

Table V is included in order to demonstrate further the reproducibility of certain analyses as obtained by the use of different aliquots of the same prepared sample of tap water. Excellent agreement in the results for the elements listed is shown. Indeed, the reproducibilities of results for the ranges of concentration listed in Tables IV and V compare favorably with those obtained by the most sensitive chemical methods, and in some cases they are far superior.

Since the flame gives rise to the low-temperature lines typical of atoms or molecules in the lowest states of excitation, the spectra produced are very simple, being characterized by a minimum of masking of important analytical lines. This is particularly favorable in connection with the choice of a spectrograph, since it permits the use of a smaller, less expensive instrument possessing a dispersion sufficient merely to separate the manganese 4031 Å. lines from the potassium doublet at 4044/7 Å. In addition to the saving effected in the purchase of suitable equipment, further economies occur in the operation of the method itself due to the low cost of acetylene and the elimination of electrodes which are very expensive when spectroscopically pure.

The air-acetylene flame is characterized by the ease and accuracy with which it is possible to reproduce the conditions of excitation and by the comparative freedom from reactions between the various ions carried by the spray into the flame (5). The latter point is of distinct advantage in accurate spectrochemical analysis, since in most applications it eliminates the need for the use of buffer salts to ensure conformity in composition of samples and the standards used to derive the analytical curves. Therefore a single curve derived from water solutions of salts of the elements of interest can be applied to the analysis of many materials which vary widely in inorganic salt composition. This is true, however, only if the method of photometry accurately corrects for the background intensity. Such accurate corrections of background are obtained by the method of photometry employing internal standard lines and intensity calibration standards, when used in the manner described above.

The absence of reactions between the cations and anions of a sample in the flame simplifies the chemical procedures needed to prepare samples for analysis. Inorganic material and biological ash may be placed in solution by the use of dilute solutions of hydrochloric, nitric, or sulfuric acid, or by water alone (5). However, in order to protect the platinum components of the equipment, mixtures of hydrochloric and nitric acids must be avoided. In the case of fluid biological material, it is frequently possible to analyze the material without any preparatory chemical treatment. This is particularly true of samples of urine in which only 1 ml. of fresh urine, provided with the internal stand-

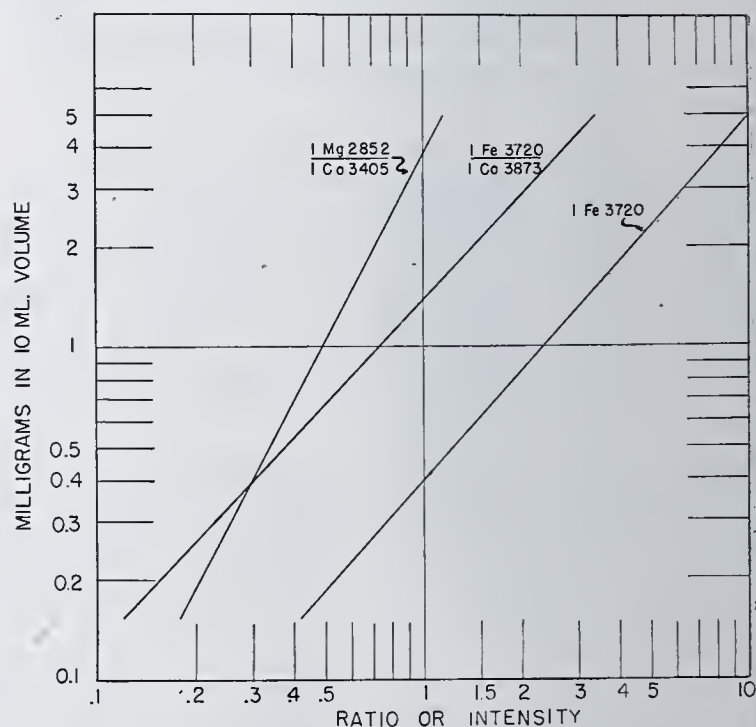


Figure 6. Typical Analytical Curves Obtained by Use of Internal Standards and Plate Calibration Procedures



Table VII. Applicability, Methods of Preparation, and Dilutions Required for Flame Analysis

Material	Amount of Sample	Preparation	Final Volume and Dilutions Mi.	Exposure Min.	Cation Determined
Tap water	500 ml.	Evaporate to dryness, dissolve in HCl and H <sub>2</sub> O	10 × 100	2 2	Mg, Na, K, Sr, Fe, Cu, Mn Ca
Inorganic salts	0.1-1.0 g.	H <sub>2</sub> O or H <sub>2</sub> O + acid solution, evaporate to volume	10	2	34 elements in proper concentration
Whole blood	2 ml.	H <sub>2</sub> SO <sub>4</sub> -HNO <sub>3</sub> -HClO <sub>4</sub> digestion	10 × 1	4 2	Mg, Na, Fe Na, Fe, K, Ca
Blood serum or plasma	2 ml.	H <sub>2</sub> SO <sub>4</sub> -HNO <sub>3</sub> -HClO <sub>4</sub> digestion	10 × 1	4 2	Mg, K, Na Ca, K
Tissue samples	5 g.	H <sub>2</sub> SO <sub>4</sub> -HNO <sub>3</sub> -HClO <sub>4</sub> digestion	10 × 1	2 2	Mg, Fe, Cu, Ca, Na K
Fruit and vegetable juices	50 g. or ml.	H <sub>2</sub> SO <sub>4</sub> -HNO <sub>3</sub> -HClO <sub>4</sub> or HNO <sub>3</sub> -H <sub>2</sub> O <sub>2</sub> digestion	10 × 50	2 2	Mg, Cu, Fe, Mn, Sr, Na Na, K, Ca
Individual food	20-50 g.	H <sub>2</sub> SO <sub>4</sub> -HNO <sub>3</sub> -HClO <sub>4</sub> or HNO <sub>3</sub> -H <sub>2</sub> O <sub>2</sub> digestion	10 × 20	2 2	Mg, Cu, Mn, Fe, Na, K, Ca Ca, Na, K, Mg
Soils (exchangeable base)	5 g.	Leach with H <sub>2</sub> O or N NH <sub>4</sub> Ac, evaporate to dryness, H <sub>2</sub> O <sub>2</sub> to destroy organic matter	25 cc. × 50	2 2	Mg, Mn, Sr, K, Na, Fe, etc. Ca
Alloys	0.1-1.0 g.	H <sub>2</sub> O + acid, evaporate to volume	10 × ?	2 2	Any of 34 in proper concentration More abundant element

Table VIII. Typical Analyses with the Air-Acetylene Flame

Material	Mg	Cu	Na	Fe	Mn	K	Ca	Sr
<i>Grams per 1000 grams or ml.</i>								
Tapwater	0.0049	....	0.010	0.00011	.....	0.0016	0.030	0.00013
Urine (human)	0.1000	....	3.90	.....	.....	2.200	0.035	.....
Orange juice (canned)	0.052	....	0.017	0.0024	0.00028	2.450	0.090	0.00022
Grape-fruit juice (canned)	0.054	....	0.0042	0.0010	0.00044	1.300	0.053	0.00050
Tomato juice (canned)	0.086	0.0004	3.075	0.0022	0.00094	3.050	0.00056	.....
String beans (canned)	0.155	....	1.600	0.0120	0.00155	1.400	0.210	<0.0001
<i>Grams per 100 grams</i>								
Whole blood, rabbit 1	0.002	....	0.150	0.030	.....	0.150	0.0090	.....
Whole blood, rabbit 2	0.006	....	0.165	0.045	.....	0.200	0.0073	.....
Whole blood, rabbit 3	0.0043	....	0.175	0.0475	.....	0.200	0.0068	.....
Blood plasma, rabbit 1	0.0018	....	0.400	.....	.....	0.016	0.019	.....
Liver (rabbit)	0.0134	....	0.140	0.0185	0.00018	0.260	0.016	.....
Liver (human)	0.0166	....	0.120	0.0096	0.0002	0.250	0.0073	.....
Brain (human)	0.0180	....	0.120	0.0052	.....	0.380	0.0073	.....
Kidney (rabbit)	0.0134	....	0.150	0.0088	0.00011	0.340	0.0050	.....

and diluted to 10 ml., suffices for the analysis of the alkalis. The accuracy of analysis in this case is illustrated in Table VI, where the results obtained by the use of the untreated sample are compared with the results obtained by the analysis following ashing of the organic materials by three distinctly different procedures. Direct analysis has also been applied to milk samples (5), and it is probable that it can be adapted to laked blood, blood serum, and blood plasma.

When the organic matrix of biological material must be destroyed, dry-ashing is satisfactory except in the case of sodium, potassium, and calcium where, as can be seen from Table VI, the results are lower than those obtained following ashing by other procedures. Although the wet-ashing technique is decidedly superior, a number of difficulties occur which force the analyst to vary the technique to fit the different conditions encountered. In the case of biological material other than bone, organic matter can be destroyed by sulfuric and nitric acids, supplemented by the use of perchloric acid. In these cases all the salts will remain in solution when the digest is diluted to the desired volume or can be made to go into solution by addition of a little hydrochloric acid and application of heat. When material contains much calcium, organic matter is destroyed by the use of nitric acid and Superoxyl. This is a more tedious procedure than the technique employing sulfuric acid, but it has the advantage of producing residues which in most cases are readily soluble in water or in dilute acids. For the detection of minor elements, it may also be necessary further to concentrate the prepared samples by evaporation, but this is limited to the point at which crystallization of salts occurs. The removal of these salts or other insoluble matter (silica) by filtration is not a safe procedure, since many elements may be lost through occlusion or adsorption. Precipitates are not particularly inimical to accurate analysis except when they are so heavy that they tend to clog the orifices of the equipment (5), or when they cannot be dispersed uniformly in the mist. Apparently it is not necessary for all the

organic matter to be destroyed (Table VI), and therefore analyses can be made on biological material in which the ashing process has been continued only until the sample can be placed in solution or dispersed finely and uniformly in the desired volume.

Since the means of preparing samples may vary widely, specific details are not given and the analyst must choose modifications which best suit his purpose, on the basis of his own experience. However, the general methods found satisfactory for various materials are indicated in Table VII, which also illustrates the size of sample employed, the dilution required for the determination of the elements, and the wide applicability of the flame method. In general, when acids are used, an attempt is made to keep their concentration at about 10% of the total volume of sample, but this may vary somewhat without affecting the results. The authors have applied the method mainly to the analysis of biological material.

Data on its applicability to other materials may be obtained from papers by Mitchell and Robertson (10) and Ells and Marshall (3), who applied the technique to the determination of exchangeable base in soil samples. The purpose of the ammonium acetate leach indicated in Table VII is to prevent the removal of large amounts of aluminum (3), high concentrations of which are said to interfere in the determination of calcium and strontium (10). The interference is said to be due to the depression of the intensities of the strontium and calcium lines and this interionic action appears to be the only one noted in the literature. Ells (2) states that the interference occurs when aluminum is present in a concentration exceeding 1 mg. per liter. Examples of the application of the method to the analysis of inorganic salts and alloys may be obtained from the paper by McClelland and Whalley (8). These investigators routinely determined calcium and sodium in magnesium and aluminum powders and alloys, as well as copper, manganese, and magnesium in aluminum. They also used the method to determine calcium, potassium, and sodium in blast-furnace and lime-kiln dusts, as well as in various inorganic salts.

For the purpose of easy computation of results, analyses are always made on a 5- or 10-ml. aliquot of each sample. This volume should include the proper amount of cobalt (5 mg. per 10 ml.) which furnishes the internal standard lines. Exposure periods may vary from 2 to 10 minutes. In the internal standard line method of photometry, as used in this work, the intensity ratios do not show significant variations with the duration of the exposure, and therefore the concentration values may be read directly from the calibration curve. By increasing the period of exposure it is also possible to detect minor elements present in amounts below the lower limit of the calibration curve merely by extrapolating the line to lower limits.

Concentrated samples are flamed first in order to detect the minor elements, and then diluted with a water solution of cobalt chloride (5 mg. of cobalt per 10 ml.) as indicated in Table VII for the determination of the more abundant elements. Between samples, the atomizer and burner are cleaned by spraying distilled water through the system for 1 to 2 minutes. Two and 4-minute exposures are employed, without the preliminary stabilizing period which is customary with the L/H method of photometry. Air pressures of 40 pounds per square inch and acetylene pressures of 25 cm. are not the most suitable conditions for the detection of minute traces of calcium and strontium, but high sensitivity is not required for the former, which is normally present in abundance, while the latter can be detected in very



small amounts by proper concentration of the sample (Table VII). The sodium doublet at 3302/3 Å. is not so sensitive as the 5890/5.9 Å. doublet, but it is satisfactory for the analysis of most biological materials and is not affected appreciably by small contaminations. The yellow sodium doublet at 5890/5.9 Å. is so sensitive that its use requires special precautions in order to prevent contamination by sodium from glassware.

In Table VIII are listed typical results obtained with various materials. In a number of instances minor elements known to be present have not been detected. This can be remedied by the use of larger samples or by employing longer periods of exposure than were used in the examples cited. Owing to the scarcity of suitable analytical lines in the region from 4607 to 6700 Å., internal standard methods in this region have not yet been developed. Of the prominent lines in this region, the barium line at 5535.5 Å. was not considered because it is too diffuse, while the strong gadolinium line at 5696 Å. (5) could not be tested because of lack of a suitable salt of this rare element. Analysis in this region (lithium particularly) at present is carried out by employing the L/H method in which the L/H values are read from a curve plotted from the ratios obtained for standard solutions taken on the same plate as the samples.

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## Determination of Ethyl Acetate in the Presence of Acetaldehyde

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In the determination of ethyl acetate in the presence of a large amount of acetaldehyde erratic results are due to the tendency of acetaldehyde to consume alkali in a varying and irregular manner. The method described here involves the quantitative oxidation of acetaldehyde to acetic acid carried out simultaneously with the saponification of the ester. A separate bisulfite determination of the acetaldehyde is made to correct the saponification value for the acetic acid produced from the aldehyde.

IN THE course of a research investigation, it was necessary to determine the amounts of ethyl acetate and acetaldehyde present in a mixture containing a high concentration of acetaldehyde. Since the material frequently contained polymerized aldehyde in addition to acetaldehyde and ethyl acetate, it was impossible to determine one component in the sample and estimate the other by difference.

Methods for estimating esters and aldehydes in such materials as distilled liquors are well known (1). The esters are determined by saponification, while the aldehydes are determined colorimetrically with sulfite fuchsin solution or volumetrically with sodium bisulfite (2). The determination of acetaldehyde in the samples under consideration presented no difficulty; the Kolthoff and Furman modification (7) of the Ripper (9) bisulfite addition method gave accurate results.

While the determination of ethyl acetate in the presence of acetaldehyde by saponification is satisfactory in cases of low concentrations of acetaldehyde (3), erratic results were frequently noted in the present study where the concentrations of the two components were much greater and where the ratio of aldehyde to ester was high.

Since the source of the error was traced to the tendency of acetaldehyde to consume alkali in a varying and irregular manner the successful use of the saponification reaction depended upon quantitative removal of the acetaldehyde from the solution, or quantitative conversion of the aldehyde to a form consuming alkali in a regular and reproducible manner. Because removal

of the acetaldehyde by precipitation or other means immediately available was considered to be too time-consuming, this method was not investigated. The quantitative oxidation of formaldehyde to formic acid with hydrogen peroxide in the presence of alkali has been described (6). Various references indicated that the analogous reaction for acetaldehyde also occurred (4, 5, 8). This suggested the possibility of oxidizing the acetaldehyde to acetic acid during the saponification reaction, thus allowing simultaneous determination of the acetaldehyde and ethyl acetate. In the following procedure the sum of the two constituents is determined, followed by a correction for the acetaldehyde present, determined from a sulfite precipitation value.

## PROCEDURE

Determine the density of the sample by means of a pycnometer, observing the usual precautions necessary when measuring the density of volatile liquids. Pipet 5 ml. of the mixture into 100-ml. volumetric flask, make up to the mark with distilled water, and mix thoroughly.

ACETALDEHYDE DETERMINATION. Pipet 5 ml. of the dilute sample into 50 cc. of 0.1 N sodium bisulfite solution contained in a 250-ml. glass-stoppered iodine flask. (This solution should contain 5 to 10% of ethyl alcohol and should be standardized daily.) Shake the sample intermittently for about 30 minutes; then wash the neck of the flask with distilled water and add a amount of standard iodine solution exactly equivalent to the sodium bisulfite. Titrate the excess iodine with standard sodium thiosulfate solution, using starch indicator near the end point.

Table I. Oxidation of Acetaldehyde to Acetic Acid

Sample No.	Acetaldehyde Present Milliequivalents	Acetic Acid Produced <sup>a</sup> Milliequivalents	Error %
1	0.43	0.43	0.0
2	0.86	0.84	2.3
3	1.29	1.27	1.6
4	1.72	1.74	1.2
			Av. 1.3

<sup>a</sup> Corrected for free acetic acid in acetaldehyde.



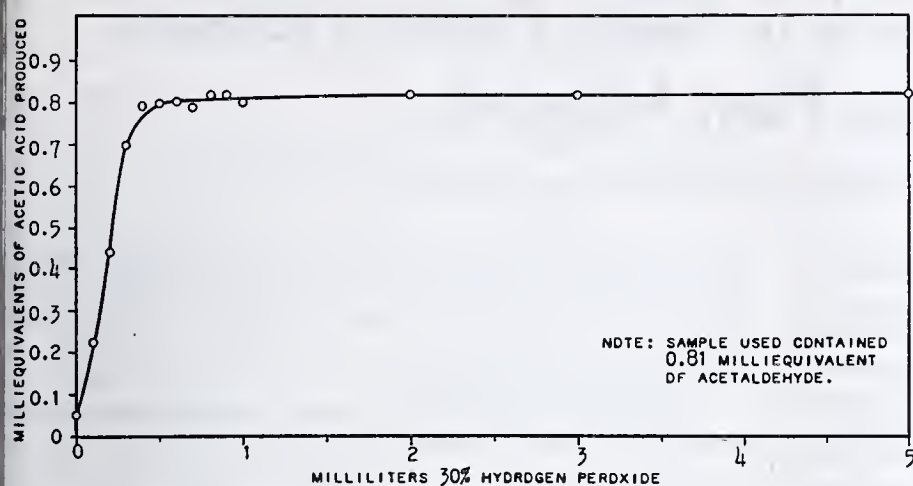


Figure 1. Effect of Excess Hydrogen Peroxide in Oxidation of Acetaldehyde to Acetic Acid

Table II. Determination of Ethyl Acetate and Acetaldehyde

Sample No.	Acet-aldehyde Added Milliequivalents	Ethyl Acetate Added Milliequivalents	Acet-aldehyde in Mixture %	Ethyl Acetate in Mixture %	Sum of Two Components Milliequivalents	Sum Found by Analysis Milliequivalents	Ethyl Acetate Found Milliequivalents	Error in Ethyl Acetate Determination %
1	0.52	3.83	12	88	4.35	4.31	3.79	-1.04
2	1.03	3.83	21	79	4.86	4.90	3.87	+1.04
3	1.03	3.08	25	75	4.11	4.13	3.10	+0.65
4	1.03	2.31	31	69	3.34	3.39	2.36	+2.16
5	1.03	1.54	40	60	2.57	2.55	1.52	-1.30
6	2.06	1.54	57	43	3.60	3.55	1.49	-3.24
Av. 1.57								

The number of milliequivalents of acetaldehyde is calculated as follows:

$$\text{Milliequivalents of acetaldehyde} = \frac{\text{ml. of Na}_2\text{S}_2\text{O}_3 \times N \text{ Na}_2\text{S}_2\text{O}_3}{\text{ml. of sample}}$$

**ETHYL ACETATE DETERMINATION.** Pipet 5 ml. of the diluted sample into an iodine flask containing approximately 100 ml. of water, 25 ml. of 0.5 *N* sodium hydroxide, and 5 ml. of 30% hydrogen peroxide. Secure the stopper of the flask, place the flask on a steam hot plate, and heat 15 minutes. (Samples reach a temperature of approximately 80° C. and a maximum pressure of about 360 mm. of mercury.) Remove the flask and allow it to stand for one hour. At the end of this time carefully open the flask, and wash the neck with distilled water. Titrate the excess alkali with 0.5 *N* hydrochloric acid, using phenolphthalein as indicator. Run a blank determination concurrently with the samples.

$$\% \text{ ethyl acetate} = \frac{\text{milliequivalents of alkali consumed} - \text{milliequivalents of acetaldehyde present} \times 0.088 \times 100}{0.25 \times \text{density of sample}}$$

#### DISCUSSION

**OXIDATION OF ACETALDEHYDE TO ACETIC ACID.** In order to study the effect of varying amounts of hydrogen peroxide on the oxidation of acetaldehyde, the following experiments were undertaken:

One-milliliter samples of a standard solution of acetaldehyde (0.81 milliequivalent per ml.) were added to flasks containing approximately 100 ml. of water, 25 ml. of 0.5 *N* sodium hydroxide, and from 0 to 5 ml. of 30% hydrogen peroxide. After heating as described in the procedure, the remaining alkali was titrated with 0.5 *N* hydrochloric acid; thus the amount of acetic acid produced was found.

Results are shown in Figure 1. Even when no hydrogen peroxide is present, a slight amount of acid is produced. This means that the acetaldehyde is oxidized by air under the conditions of testing, since a correction was made for the small amount of acetic acid initially present in the acetaldehyde. Apparently

no deleterious effect is produced by a large excess of hydrogen peroxide, and, since Figure 1 shows that more than a stoichiometric relationship is necessary, it is advisable to maintain an excess. This excess is ensured by the amount recommended in the procedure.

After determination of the quantity of hydrogen peroxide required for complete oxidation of acetaldehyde, samples containing varying amounts of aldehyde were prepared and the amount of acetic acid produced was determined. Table I shows the quantitative nature of the oxidation.

Experiments were also carried out to determine whether there would be any oxidation of the ethyl alcohol produced in the saponification. No appreciable amount of acid was produced when alkaline solutions of ethyl alcohol were treated with varying amounts of hydrogen peroxide, and it was concluded that this component would cause no errors in the method.

#### RESULTS

To test the application of the method to various mixtures of ethyl acetate and acetaldehyde, solutions were prepared as discussed below and analyzed according to the procedure previously described. The accuracy of the method may be judged by data in Table II.

**ACETALDEHYDE SOLUTION.** A standard acetaldehyde solution was prepared by diluting 12 to 15 ml. of chilled redistilled acetaldehyde to 250 ml. with chilled carbonate-free distilled water. This solution was standardized against the bisulfite solution by the method described above.

**ETHYL ACETATE SOLUTION.** A standard ethyl acetate solution was prepared by diluting 18 to 20 ml. of redistilled ethyl acetate to 250 ml. with carbonate-free distilled water. The amount of ester in terms of milliequivalents per ml. was determined.

#### SUMMARY

The reaction of acetaldehyde with alkali during the saponification of ethyl acetate has been noted in mixtures of these two compounds. A method eliminating the erratic results so produced involves oxidation of the aldehyde with hydrogen peroxide in alkaline solution. Saponification data are corrected for the effect of the acetic acid produced by the oxidation reaction, by a separate bisulfite determination of the acetaldehyde. Average accuracy within 2% is reported.

#### ACKNOWLEDGMENT

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# Determination of Starch in Sweet Potato Products and Other Plant Materials

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A polarimetric method has been developed for the determination of starch in sweet potato products and other plant materials. By use of the specific and quantitative precipitation of starch as starch iodide and of uranyl acetate as a protein precipitant, the effect of substances which interfere with most methods for the determination of starch has been largely eliminated. When the method was tested on samples containing large amounts of protein, pectin, inulin, and other interfering substances, the starch values obtained were closer to the true starch content than were found by either the malt-diastase or Hopkins method. The addition of a number of different substances commonly found in biological materials did not significantly alter the starch values obtained with the proposed method. The procedure described should be applicable to materials containing 10% or more starch on the moisture-free basis. The specific rotation for the conversion of polarimetric readings to starch was found to be 200.9 for the proposed method.

MANY of the methods currently available for the determination of starch are limited in application because they fail to give true starch values when the sample contains pectin, non-starch polysaccharides, proteins, and other interfering substances. With sweet potato material it is especially important to eliminate the interference of pectin. In developing the proposed method two principles brought out by the work of Denny (4) have been followed. (1) A starch method to be generally applicable must give correct values when pectin, nonstarch polysaccharides, and proteins are present in the sample. (2) The method should give zero or very low values on samples containing little or no starch by qualitative tests, but containing high amounts of substances which might possibly interfere. Parts of the methods of Hopkins (?), Denny (5), Pucher and Vickery (9), and Sullivan (10) have been used in the proposed method. Only the procedure finally adopted and data to establish its validity are given here.

The proposed method consists of treatment of the sample at boiling temperature with dilute ammonium carbonate solution, precipitation of the starch with iodine, decomposition of the starch iodide, reprecipitation with iodine, decomposition of the starch iodide, precipitation of the starch with alcohol, dispersion in calcium chloride, precipitation of any remaining protein with uranyl acetate, and determination of the optical rotation. The method is recommended for samples containing 10% or more starch on the moisture-free basis.

## REAGENTS

Celite, an analytical filter aid.

Ammonium Carbonate Solution. Dissolve 3.0 grams of reagent quality ammonium carbonate in water and dilute to 1 liter.

Sodium Chloride Solution. Dissolve 200 grams of reagent quality sodium chloride in water and dilute to 1 liter.

Iodine-Potassium Iodide Solution. Dissolve 30 grams of iodine and 50 grams of potassium iodide in water and dilute to 250 ml.

Sodium Thiosulfate Solution. Dissolve 125 grams of sodium thiosulfate pentahydrate in water and dilute to 1 liter.

Hydrochloric Acid, approximately *N*.

Ethyl Alcohol, 95% by volume.

Ethyl Alcohol, 70% by volume.

Calcium Chloride Solution, 2 parts of crystalline calcium chloride hexahydrate plus one part of water. Adjust to a density of 1.30, make very faintly pink to phenolphthalein with 0.1 *N* so-

dium hydroxide and filter. Because of the effect of the high concentration of calcium chloride, the reagent prepared in this manner has a lower pH than is usually indicated by the phenolphthalein end point.

Octyl Alcohol, octanol-1.

Acetic Acid, 0.8% solution.

Uranyl Acetate, 5% aqueous solution. Prepare every 3 or 4 days from pulverized reagent.

## ANALYTICAL PROCEDURE

Weigh accurately 0.9 to 1.0 gram of the finely ground sample, 80-mesh or finer, into a 100-ml. heavy Pyrex centrifuge tube (30 by 170 mm., without pourout) and add 2.0 grams of Celite. Add 50 ml. of ammonium carbonate solution and stir with a strong glass rod until the material is completely wetted. Place in an oil bath maintained at 117-120° C. and boil for 30 minutes, adding a drop or two of octyl alcohol to prevent excessive foaming, if necessary. Stir the suspension frequently during the 30 minutes' boiling. If excessive foaming persists after the addition of octyl alcohol, it may be controlled by raising the tube so that it does not extend so deeply into the bath. After the initial foaming has subsided, the tube should be lowered so that vigorous boiling is resumed.

Cool to room temperature, rinse off the stirring rod, and place it aside for subsequent use with the same sample. Add 20 ml. of sodium chloride solution and 2.5 ml. of the iodine-potassium iodide solution. Almost fill the tube with water and stopper with a tightly fitting rubber stopper. Invert and shake gently, so that all the starch reacts with the iodine. Precipitation usually occurs in about 5 minutes. Let the solution stand for at least 5 minutes after precipitation starts. Remove stopper and wash any adhering starch iodide into the tube with small quantities of water from a wash bottle. Centrifuge at about 2000 r.p.m. for 10 minutes.

Pour off and discard the supernatant liquid. Add 20 ml. of the 20% sodium chloride solution to the precipitate. Suspend the starch iodide with the glass rod, previously set aside, and add sodium thiosulfate solution until the starch iodide is decomposed. A long, thin, flexible spatula is more convenient for suspending the starch iodide than the glass rod. The glass rod should be saved, however, for the final dispersion of the starch. About 3.5 ml. of the thiosulfate solution will be required on relatively pure starches and excess should be avoided.

Add 10 ml. of *N* hydrochloric acid, 2.5 ml. of iodine-potassium iodide solution, and enough water to fill the tube. Restopper and shake gently. After the starch iodide has precipitated centrifuge as before and discard the supernatant liquid. Add approximately 50 ml. of 95% ethyl alcohol and thoroughly suspend the starch iodide. Add sodium thiosulfate solution until the blue color is discharged, avoiding excess, since too much may cause cloudiness in the final dispersion. Add water to make approximately 70% alcohol, stopper, and shake thoroughly. Allow to stand about 15 minutes and centrifuge 10 minutes. Wash the precipitated starch once with 50 ml. of 70% alcohol by volume. Add a total of 60 ml. of concentrated calcium chloride solution, a small amount at a time, and stir with the rod previously set aside, until the material is suspended and free from lumps. Add 3 ml. of 0.8% acetic acid, place in the oil bath, and boil for 18 minutes. A shorter period of boiling may lead to cloudiness in the final solution, while a longer period may give values which are slightly low. Raise the tube and while still hot add 5 ml. of 5% uranyl acetate solution and stir well.

Transfer the contents of the tube with water to a 100-ml. volumetric flask, cool to room temperature, make to volume add 1 ml. of water to correct for volume occupied by Celite, and shake thoroughly. A small correction for the volume occupied by tissue residue could also be made here. Transfer to a dry centrifuge tube and centrifuge for 10 minutes at 2000 r.p.m. Protect the solutions against evaporation by use of rubber caps. Occasionally some floating material is present in the supernatant liquid and can be removed readily by filtering through Whatman No. 4 paper or an equivalent fast paper.

Place the supernatant or filtrate in either a 2- or a 4-dm polarimeter tube, depending on clarity, and read in a polar



imeter at about 25° C. using the sodium D line. Take 10 readings, approaching the match point alternately from each side.

Calculate the starch content by the following equation:

$$\text{Percentage of starch} = \frac{\text{observed rotation} \times 100 \times 100}{\text{tube length in dm.} \times 200.9 \times \text{wt. of sample in grams}}$$

#### DISCUSSION OF ANALYTICAL PROCEDURE

The material undergoing analysis is boiled first with ammonium carbonate solution to neutralize the natural acidity of plant material. It may be necessary to increase the ammonium carbonate concentration for excessively acid samples. The treatment with ammonium carbonate also disperses or dissolves the pectins, sugars, soluble proteins, etc., which are separated from the starch iodide by centrifuging and decantation. The decomposition of the starch iodide and second precipitation with iodine liberate impurities which may have been carried down with the first starch iodide precipitate. After the last addition of thiosulfate, 70% alcohol is used to remove excess sodium thiosulfate and materials not soluble in water but soluble in 70% alcohol. This step promotes clarity of the final solution. Calcium chloride is used in preference to other solubilizing agents because of its ability to disperse starch readily and its use in other methods (3, 5-10). Starch dispersed in calcium chloride solution does not degrade appreciably even on long standing. Solutions that had stood 24 to 48 hours showed no significant changes in rotations. Starch solutions which had been stored in glass-stoppered containers for 30 to 90 days showed an increased dextrorotatory power due to evaporation. The acidity during solubilization with calcium chloride is somewhat greater than that recommended by Hopkins (7) and Mannich and Lenz (8). This increased acidity appears to have no adverse effect on the starch and promotes greater clarity. Uranyl acetate is added at the end to remove any proteins not removed by previous treatment. Other protein precipitants were tried but proved unsatisfactory. The use of uranyl acetate as a protein precipitant will probably prove useful in the Hopkins method and in other polarimetric methods for starch.

In determining the starch content in cornstarch, wheat starch, and waxy maize starch, the final solution was usually too cloudy to read in a 4-dm. tube. Shaking the solution vigorously with about 10 ml. of carbon tetrachloride, centrifuging, and decanting the starch dispersion gave clear solutions. Chloroform may be used in place of carbon tetrachloride, but in this case correction must be made for the solubility of chloroform in the starch dispersion.

The method can probably be shortened when used with materials known to be relatively free of pectin and protein by making only one precipitation with iodine.

#### DETERMINATION OF FACTOR FOR CONVERSION OF POLARIMETRIC READINGS TO STARCH

Since pure starch or starch of known purity has probably never been prepared, it is not possible to determine accurately the specific rotation,  $[\alpha]_D$ , of starch by dissolving a weighed sample and reading it in a polarimeter. It is, however, possible to estimate the specific rotation of starch by determining the amounts of the major impurities, either before or after purification of the starch and assuming that the rest of the sample is starch. It is also possible to estimate the factor by assuming some other starch method to be correct and using the value so obtained for calculating the specific rotation. Mannich and Lenz (8) and Hopkins (7) recommended the use of +200 for the specific rotation of wheat starch. However, since the value varies somewhat with the method, depending on the degree of degradation of the starch, it was necessary to determine the proper factor to use with the proposed method.

The first procedure used to arrive at the factor for the method was to determine the nonstarch material in the starch samples

Table I. Estimation of Factor by Correction for Major Impurities

Kind of Starch	Nonstarch					Starch (by Difference)	$[\alpha]_D$ (Calculated)
	Moisture %	Ash %	Protein %	Extractives %	Total %		
Sweet potato A	12.52	0.17	0.08	0.21	12.98	87.02	199.5
Sweet potato B	12.01	0.36	0.05	0.26	12.68	87.32	198.9
Corn	12.44	0.07	0.21	0.45	13.17	86.83	198.5
Wheat	12.45	0.11	0.27	0.41	13.24	86.76	196.4
Waxy maize	11.99	0.10	0.25	0.19	12.53	87.47	197.7

Table II. Estimation of Factor by Use of Starch Put through Proposed Method

Kind of Starch	Ash %	Moisture %	Starch (by Difference) %	$[\alpha]_D$ (Calculated)
Sweet potato B	0.52	13.63	85.85	200.1
Corn	2.94	12.10	84.96	201.0
Wheat	1.53	10.44	88.03	201.9
Waxy maize	0.32	11.48	88.20	200.6

under investigation. Ash was determined by ignition at 550° C. for 3 hours. Nitrogen was determined by the Kjeldahl procedure, followed by nesslerization of the distillate. Protein was calculated by use of the appropriate factor. Extractives were obtained by a 16-hour extraction with 85% methanol followed by a 5-hour extraction with ethyl ether. Moisture values were calculated from the loss in weight of samples dried approximately 16 hours at 96° to 100° C. in a vacuum oven equipped with a drying train. The results are shown in Table I.

The value for the specific rotations of sweet potato, corn, and waxy maize starches put through the proposed method is approximately 199 when estimated by this procedure. Wheat starch gave a lower value, which may be due to the presence of some impurities which were not accounted for.

The second procedure for arriving at the factor was to assume that the malt-diastase method gives correct values on reasonably pure starches when the 0.93 factor is used to convert glucose to starch. On this basis, the specific rotations found with the proposed method were 202.0, 200.6, 200.3, 198.4, and 199.9, respectively, for starches in the order listed in Table I.

The third procedure for obtaining the factor for the method was to use the starch obtained from the final calcium chloride solution.

Eight 2.0-gram samples of each starch were put through the method and the combined final solution was dialyzed, in Visking sausage casing, against running distilled water for 2 days to remove the calcium chloride and other salts. After concentration of the solution by pervaporation the starch was precipitated with an alcohol-ether mixture and washed three times with ethyl alcohol and twice with ethyl ether. The precipitated starch was dried in vacuo over sulfuric acid. After air-equilibration, moisture and ash were determined. Specific rotation was then found by dissolving a sample in calcium chloride solution, using just sufficient heat to ensure complete dispersion. The materials dispersed readily and the preparation from waxy maize dispersed in the cold. The addition of dilute acetic acid was unnecessary. The results are given in Table II.

The calculated values for the specific rotation are now in closer agreement and wheat starch shows less divergence from the other starches. The average specific rotation found for the starches by this procedure is 200.9. Since this procedure gives slightly higher and more consistent values than the first procedure, the value 200.9 has been selected for use with the proposed method. The average value of 200.2 found by assuming the correctness of the malt-diastase method, with 0.93 factor, is supporting evidence. The value 200.9 is probably a close approximation for the value of the specific rotation of the starch as it exists at the time of polarization in the proposed method.

#### COMPARISON WITH MALT-DIASTASE AND HOPKINS PROCEDURES

The proposed method was compared with the official A.O.A.C. malt-diastase method for starch (1, p. 359), the factor 0.93 (2)



Table III. Comparison of Methods for Determination of Starch

Sample No.	Kind of Material	Per Cent Starch on Moisture-Free Basis			
		Malt-diastase	Hopkins, 200 factor	Hopkins, 204.8 factor	Proposed method
1	Sweet potato starch A	98.2	100.9	98.5	98.8
2	Sweet potato starch B	98.4	100.5	98.1	98.0
3	Waxy maize starch	98.3	100.4	98.0	97.9
4	Cornstarch	98.2	100.1	97.8	97.8
5	Wheat starch	98.1	99.1	96.7	96.9
6	Ground corn	70.5	74.2	72.5	71.6
7	Bread	67.4	68.2	66.6	64.6
8	Sweet potato residual pulp, water process, dried	55.5	56.4	55.1	54.8
9	Sweet potato by-product pulp, lime-water process, dried	62.1	50.4	49.2	61.4
10	Sweet potatoes, dehydrated, food type	60.5	45.9	44.8	41.9
11	Sweet potatoes, dehydrated, food type, extracted	70.8	76.1	74.3	70.3
12	Sweet potatoes, dehydrated, stock-feed type	59.1	61.8	60.4	59.3
13	Cottonseed meal	9.8	-4.4	-4.3	-0.6
14	Orange rind	12.8	22.6	22.1	-0.2
15	Jerusalem artichokes	27.4	-18.0	-17.6	-0.3
16	Peanut meal	9.1	3.5	3.4	6.7
17	Gladiolus leaves	1.3	0.3	0.3	0.4

being used instead of the official 0.90 factor to convert glucose to starch, and with the tentative Hopkins method (?), the final solution being clarified by centrifuging with Celite. The samples chosen ranged from relatively pure starches to samples containing little or no starch, but large amounts of other substances that might possibly interfere. Since stable, homogeneous samples were required for the comparison of methods, dry, finely ground materials were used. However, analysis of fresh plant materials by the proposed method should present no difficulties other than those of sampling, sample preservation, and preparation. The conventional preparation of fresh sample materials by dropping into hot alcohol (1, p. 125) is suggested. A description of the samples follows:

1. Sweet potato starch A. A.O.A.C.-1942 sample for collaborative studies on starch methods.
2. Sweet potato starch B. A laboratory-prepared starch, extracted and purified without use of lime water by the Sweet-potato Products Division of this laboratory.
3. Waxy maize starch, furnished by the American Maize-Products Company, Roby, Ind.
4. Cornstarch. A.O.A.C.-1943 sample for collaborative studies on starch methods.
5. Wheat Starch. A.O.A.C.-1943 sample.
6. Ground Corn. A.O.A.C.-1943 sample.
7. Bread. A.O.A.C.-1943 sample.
8. Sweet potato pulp. A residual pulp from water-extraction of starch without use of lime water, dried at 60° C. in a mechanical convection oven. Furnished by the Sweetpotato Products Division of this laboratory.
9. Sweet potato pulp. A dried residual pulp, typical of lime-water process by-product, from the Laurel Starch Plant, Laurel, Miss.
10. Sweet potato (dehydrated). Peeled, blanched, and dehydrated sweet potatoes, prepared for food use by the Sweet-potato Products Division of this laboratory.
11. Sweet potato (dehydrated). Same as 10 except Soxhlet-extracted with 80% alcohol before analysis.
12. Sweet potato (dehydrated). Whole sweet potato cosses, dried for the production of stock feed, from the Sweet-potato Products Division of this laboratory.
13. Cottonseed meal. Composite of cottonseed kernels, thoroughly extracted with ethyl ether before analysis.
14. Orange rind. Composite of many kinds of orange peel that had been dropped into boiling alcohol, ground in food chopper, Soxhlet extracted with 80% alcohol, dried, and ground in ball mill.
15. Jerusalem artichokes. From T. A. Kiesselbach of the University of Nebraska. Prepared for analysis in same manner as orange rind.
16. Peanut meal. Peanut kernels successively extracted with petroleum ether, ethyl ether, 95% alcohol, dried, and ground finely.
17. Gladiolus leaves, local garden variety. Dropped into boiling alcohol, dried, and ground finely.

Comparative results by the official A.O.A.C. malt-diastase Hopkins, and the proposed methods are shown in Table III.

On the relatively pure starches the malt-diastase method and the proposed method are in good agreement. The Hopkins method values (factor, 200) are higher on the starches than the values by the other two methods. This is clearly due to the use of the factor 200 for the specific rotation of starch in the Hopkins method. The factors calculated for the Hopkins method, after correcting for impurities and moisture, are 202.8, 202.6, 202.0, 200.0, and 201.8, respectively, for the starches in the order listed in Table I. If we assume the values by the proposed method to be correct, the factors calculated for the Hopkins method are 204.3, 205.2, 204.9, 204.5, and 204.9 for these starches, or an average of 204.8. Recalculation of the Hopkins values with this assumed correct value of 204.8, in order to eliminate the question of factors from the comparison, brings the starch content of the starches into good agreement by all three methods, as is shown in Table III. Earle and Milner (6) have recommended 203.0 for the factor in their modification of the Hopkins method.

On the sweet potato materials the agreement between the proposed method and the malt-diastase method is good with the exception of the sample of dehydrated sweet potatoes, sample 10. In this case the high malt-diastase values are due to the presence of sugars and other nonstarch carbohydrates. After this sample was extracted with 80% alcohol in a Soxhlet extractor to remove a large amount of sugars, including those formed during the dehydration process, the agreement was good. With the exception of the limed sweet potato pulp, sample 9, which gave low Hopkins values due to interference of the lime with the dispersion of the starch, the Hopkins method gave higher values than the proposed method on sweet potato samples. This is true even if the factor 204.8 is used instead of 200 and may be due to interference of pectin in the Hopkins method. The high value in the case of bread with the malt-diastase procedure is probably due to the presence of sugars, dextrans, or degraded starch in the sample.

The results with the orange rind, Jerusalem artichokes, gladiolus leaves, cottonseed meal, and peanut meal are of special significance with regard to the validity of the three methods. These samples were chosen because they were low in starch and high in substances that might possibly interfere. The true starch content of the orange rind sample is probably close to 0.4%. This is based on colorimetric measurements of the blue color produced with iodine. The proposed method gives a negative value of 0.2% on this sample, which is much closer to the truth than 12.8% by the malt-diastase and 22.6% by the Hopkins method. This shows that the pectin present in orange rind does not interfere with the proposed method but does interfere with the other two methods. The Jerusalem artichoke sample was starch-free by qualitative test with iodine. The proposed method gave a negative value of 0.3% on this sample, which is much closer to zero than the values of 27.4 and -18.0 found with the malt-diastase and Hopkins methods, respectively. The gladiolus leaves, which were starch-free, gave about the same values by the proposed and Hopkins methods. The cottonseed meal was used as a sample high in proteins which show negative optical rotation. Its true starch content was close to 0.2% based on colorimetric measurements. The value of -0.6% is closer to the true starch value than -4.4% by the Hopkins method. This shows that the interference of protein is small in the proposed method. The high value for cottonseed meal with the malt-diastase method is probably due to the presence of raffinose in the sample. The peanut meal contained considerable starch, 6.7% by the proposed method. As expected, the malt-diastase method gave a high value with peanut meal, probably due to the presence of nonstarch carbohydrates in the sample, and the Hopkins method gave low values due to the negative optical rotation of proteins.

It will be seen from the above comparison of methods, that in practically every case of disagreement the proposed method gave a value closer to the true value than the other two methods.

#### DETERMINATIONS MADE IN PRESENCE OF VARIOUS SUBSTANCES

The method was tested in the presence of sugars, amino acids, proteins, pentosans, pectin, and other substances often found in biological materials. In each case 200 mg. of the substance were added to an accurately weighed starch sample and the mixture was analyzed by the proposed method. The results are given in Table IV.



Table IV. Determination of Starch by the Proposed Method in the Presence of Added Substances

Substance Added <sup>a</sup>	Starch Found, Air-Dry Basis	
	Control %	Control plus substance %
Raffinose (commercial)	85.9	86.0
Sucrose (commercial)	85.9	86.3
Gum arabic (commercial)	86.5	86.5
Egg albumin (commercial)	86.5	86.6
Inulin (commercial)	86.3	86.5
Gluten (laboratory purified)	86.4	86.6
Cystine (commercial)	86.1	86.5
Proline (commercial)	86.1	86.5
White potato dextrin (commercial)	86.1	87.3
Levulose (commercial)	86.6	86.7
Maltose (commercial)	86.6	86.8
Malic acid (commercial)	86.4	86.8
Sweet potato pectin (laboratory preparation)	86.0	86.2

<sup>a</sup> 200 mg. of each substance added to 1-gram samples of sweet potato starch B.

Table V. Recovery of Added Starch<sup>a</sup>

	A Mg.	B Mg.	C Mg.	D Mg.
From 1.0 gram of cottonseed meal				
Starch added	28.1	68.7	112.1	156.5
Starch recovered	29.4	69.6	112.9	155.8
From 1.0 gram of orange rind				
Starch added	24.9	69.2	115.5	159.0
Starch recovered	26.0	70.6	118.1	158.8

<sup>a</sup> Calculated as empirically pure starch.

The only substance listed that showed evidence of interference was white potato dextrin. This material gave a red color with iodine and interfered slightly. Higher polymer dextrans, which give a violet or violet-blue color with iodine and which may be found in slightly degraded starch, do not precipitate completely when the starch is precipitated with iodine in the method and if present may be readily detected in the supernatant liquid. They are partially carried down with the starch iodide and consequently behave in an anomalous manner in the method. Consequently the results on samples containing higher dextrans are of doubtful validity. Glycogen acts in much the same way. When it is analyzed alone by the method, it is almost completely removed, but in the presence of starch it is carried down with the starch iodide and included as starch.

#### RECOVERY OF ADDED STARCH

Sweet potato starch was added in various quantities to 1.0-gram samples of cottonseed meal and orange rind and the mixture analyzed by the proposed method. Controls without starch were also run and the polarimetric readings subtracted from the polarimetric readings of the samples to which starch had been added. The results given in Table V show that the method works well in the presence of large amounts of pectin and protein.

#### PRECISION AND ACCURACY

Duplicates on 1-gram samples of relatively pure starches usually agree within 0.4%, or 4 mg. of starch. The agreement is dependent to a great extent on the precision obtainable from a polarimeter. In most instruments this precision is  $\pm 0.01$  angular degree. The deviation between duplicates, then, due to the instrument alone, for a 1-gram sample in a 4-dm. tube may be expected to be as great as 0.25%, even if the highest precision of the instrument is attained. This variation becomes increasingly important as the starch content decreases. Based on experiments with 1-gram samples containing large amounts of pectin and protein, it may be concluded that the method gives values that are within 10 mg. of the true starch content even on samples containing large quantities of the substances which interfere in most starch methods. The latter statement assumes that the factor 200.9 for the specific rotation of starch in the

final solution is essentially correct. As an illustration of the reproducibility of the method, duplicate determinations on sample 12 gave 59.3 and 59.3% starch on the dry basis, while duplicate determinations made 5 months later gave 59.8 and 59.8%. An analyst, who had not used the method previously, obtained 59.3 and 59.5% on the same sample.

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## Colorimetric Assay of Quaternary Ammonium Salts

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RECENTLY a method was described for the determination of germicidal quaternary ammonium salts in dilute solution (1). Since publication of this paper, comments from various correspondents have made it clear that some workers find it difficult to clarify—i.e., dry—the ethylene dichloride dye solution without affecting its color intensity. Re-examination of this point led to the decision to use benzene instead of ethylene dichloride. Benzene is not so good a solvent for the colored salt, but, being lighter than water, it can conveniently be clarified by centrifugation, thus avoiding all danger of contamination. At the same time, to compensate for the loss of solvent power, two other changes were made: (1) The amount of sample was reduced to one fourth—50 micrograms. (2) The light filter was changed from one transmitting at about 540  $m\mu$  to one transmitting at about 600  $m\mu$ . The method, as now used, is as follows:

In a 125-ml. Squibb separatory funnel, take 50 ml. of water containing 50 to 75 micrograms of the quaternary compound. Ordinary stopcock grease should be avoided. Starch-glycerol lubricant is satisfactory (2). Add 5 ml. of 10% sodium carbonate solution, 1 ml. of aqueous 0.04% bromophenol blue indicator solution, and exactly 10 ml. of benzene. (The indicator solution should be prepared on the day it is to be used. Dissolve 40 mg. of bromophenol blue powder in 100 ml. of water containing 1 ml. of 0.1 *N* sodium hydroxide.) Shake steadily for 2.5 to 3 minutes, let the layers separate roughly (20 to 30 seconds), and then swirl the funnel contents. Let stand several minutes or until well separated. Rinse a 15-ml. centrifuge tube with a portion of the lower aqueous layer, discard this layer entirely, and then run the colored benzene layer into the tube. Stopper the tube with a clean rubber diaphragm stopper and centrifuge for a few minutes at about 1000 r.p.m., if necessary to clarify. Transfer to a dry Klett-Summerson colorimeter tube, and read, using filter No. 60.

Changes in technique involve no change in rationale of method. Limit of error is about  $\pm 2\%$ , with occasional errors  $\pm 5\%$ .

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# Spectrophotometric Study of the Oxidation of Quenching Oils

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With the wartime development of large-scale production of aluminum alloy castings, especially for airplane motors, there has been little information available on the choice and performance of quenching oils for these alloys. In one large foundry it was found that the strain residual in quenched castings increased markedly with continued use or aging of a well-known quenching oil in the 5000-gallon tank. A spectrophotometric study has been undertaken to evaluate the changes which have occurred in this oil with an extension to a study of the rate of oxidation of several other commercial oils recommended for quenching, at high temperatures by a laboratory procedure, with and without addition agents and in the presence and absence of the aluminum alloy as catalyst. Spectrophotometric data are believed related to the light scattered by colloidal particles or precipitates as indicated by absorption curves and electron micrographs. Oxidation stability and absence of precipitable polymer particles are correlated with the quality of quenched castings as measured in terms of residual strain.

RECENTLY aluminum alloy motor castings quenched in a particular oil in a 5000-gallon tank have exhibited an increasing amount of internal strain with continued use or aging of the quenching oil. This residual strain is indicated both by x-ray diffraction patterns and by mechanical measurement of bow when strain is relieved by sawing the casting nearly through vertically. These castings of aluminum alloy were quenched rapidly from a temperature of 480° C. (900° F.) in this quenching oil maintained at approximately 45° C. This hot quenching of the metal very evidently oxidized the oil, producing a by-product which in some manner impaired the heat-conduction properties of the oil. The aluminum alloy castings seemed particularly sensitive to this change in the heat-conduction properties. It was the authors' problem to find some reliable method of studying and evaluating the deterioration of this quenching oil.

The method chosen was based on spectrophotometric values of the oil at different stages of use. It was then applied to a variety of other commercial quenching oils oxidized under laboratory conditions in order to gain some idea as to whether the optical data might indicate relative stabilities, as, of course, would be shown by a number of more familiar and accepted tests in petroleum laboratories.

## TYPE OF OILS

The results of ten commercial quenching oils are presented here. These samples were chosen from among a large number of commercial quenching oils and represent oils supposedly of the best oxidation stability available. They were essentially paraffin-base oils of light color, A.P.I. gravity near 30, and boiling largely between 300° and 400° C. Some of the quenching oil samples contained commercial polar and nonpolar additives designed to increase the stability of the oil and impart desirable heat-conduction properties. All the oils contained varying amounts of aromatic compounds of lower stability than the bulk of the oil. Table I lists the properties, as far as known, of these ten commercial quenching oils.

## PRODUCTS OF OXIDATION

Fenske (2, 3) in heating lubricating oils at 140° to 170° C. accounted for most of the oxygen absorbed. His results indicate that about half of the oxygen absorbed goes into the formation of water, while the remainder may be accounted as carbon dioxide, carbon monoxide, volatile acids, fixed acids, and isopentane-insolubles (polymers and lacquers). The isopentane-insolubles or precipitates have been classified according to solubility, color, and melting point, but there appears to be no clear line of demarcation. They probably are polymers of low molecular weight (less than 2000). Their solubility is low in isopentane and moderate in chloroform, ether, and benzene (6).

## APPARATUS AND TECHNIQUE

Throughout the history of petroleum analysis, color has been used in a qualitative sense in testing for purity. The older methods of visual comparison with colored disks or standards is gradually giving way to more reliable photometric methods utilizing standard color filters (1). Further refinements of these color tests lie in the use of prism or grating instruments capable of measuring light transmission through oils at any wave length near the visible region.

In this work, a Cenco-Sheard Spectrophotometer was used. The sensitivity below 400 mμ was low with darker colored oils. In this range with dark-colored oils an error of 5% in the transmission readings was possible. In the rest of the range an error of 2% limited the accuracy. The large slit width required in testing dark-colored oils in the ultraviolet range led to a spectral width of the transmission readings as much as 10 millimicrons. The error due to overlapping orders was small but appreciable as determined by the use of filters. In order to obtain a satisfactory

Table I. Properties of Quenching Oils

Oil	Properties
1	Commercial paraffin-base oil containing nonpolar additives
2	Commercial paraffin-base oil containing nonpolar additives
3	Highly refined, paraffin-base, colorless oil, containing no additives
4	Experimental straight-run quenching oil without any additives
5	Paraffin-base oil for commercial quenching oils
6	Oil 5 containing 0.85% of lard oil and 0.3% of polar additives
7	Experimental paraffin-base quenching oil containing additives
8	Similar to oil 7
9	Pure, colorless, light paraffin oil
10	Commercial lubricating oil containing special polar addition agents

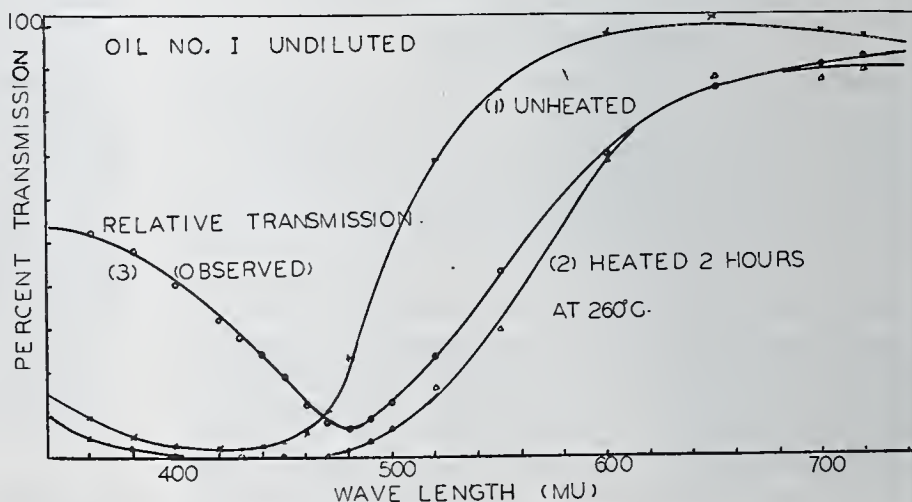


Figure 1. Transmission vs. Wave Length



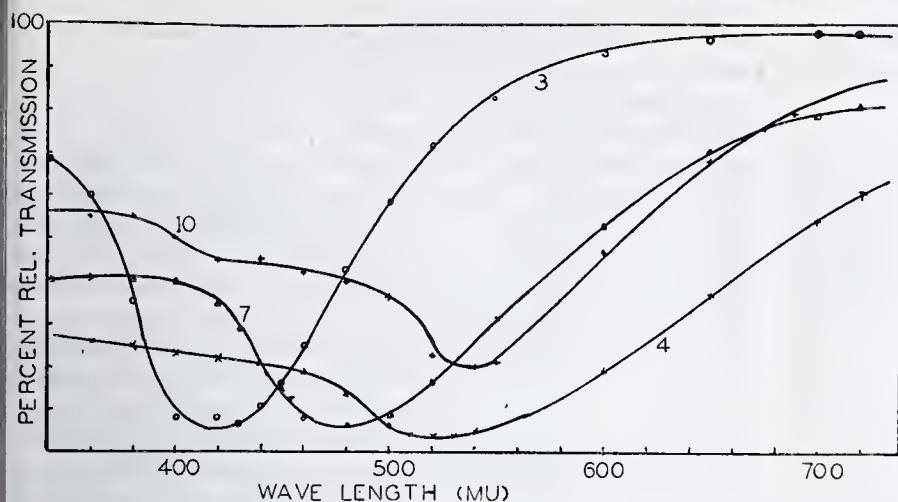


Figure 2. Relative Transmission vs. Wave Length  
Commercial quenching oils heated 2 hours at 260° C. (undiluted)

Table II. Transmission vs. Wave Length

(Oil 1 heated at 260° C. for 2 hours, undiluted)

Wave Length	$T_1$ Unheated	$T_2$ Heated	$T_1/T_2$ (Calculated)	$T_1/T_2$ (Observed)
340	15	10	66	52
360	9.0	4.5	50	52
380	4.5	2.5	55	48
400	3.0	1.3	43	40
420	3.0	1.0	33	32
430	3.0	1.0	33	28
440	3.0	0.8	27	24
450	3.7	0.8	22	19
460	6.0	0.8	13	12
470	...	...	...	8
480	28	1.6	5.7	7
490	...	...	...	9
500	49	6.5	13	13
520	69	17	25	24
550	85	30	35	43
600	98	69	70	70
650	102	88	86	85
700	98	87	89	91
720	97	90	93	92

accuracy with dark-colored oils it was necessary to dilute the samples. Petroleum ether was chosen as a cheap, easily handled solvent.

#### EXPERIMENTAL CURVES AND NUMERICAL RATING OF OILS

If the transmission vs. wave-length values for a typical quenching oil are plotted, curve 1 in Figure 1 is obtained. Curve 2 represents the spectral curve for the same oil heated 2 hours at 260° C. If the ratio quotient of these two curves is plotted with the transmission value of the unheated oil at 100 we obtain a curve similar to function 3 on the graph. Table II lists the calculated and observed values for these relative transmissions. The value of the relative transmission curves lies in their evaluation of the oxidation products formed in the oil. The exact nature of the oxidation products producing these curves is unknown, though thought to be tied up with the formation of the polymer "precipitates".

These relative transmission curves are observed to vary widely from oil to oil in the position and character of the absorption peak (minimum transmission), as can be seen in Figure 2 in which several commercial quenching oils are graphically compared. In general, the more highly refined oils show absorption peaks further toward the violet than do oils containing aromatic compounds. This shift in absorption peaks is misleading in oils which are evaluated on the basis of visible color only. An oxidized oil of light color may actually contain more oxidation and polymer products than a dark-

colored oil similarly treated. Under some conditions, usually oils heated at relatively low temperatures (150° C.), abnormal curves may be obtained which show several relative absorption peaks occurring after heating for various lengths of time.

#### DILUTION EFFECTS

When working with dark-colored oils or light-colored oils having a large absorption in the near ultraviolet region, it is necessary to dilute the samples with a neutral solvent to obtain a maximum sensitivity. A high-boiling petroleum ether was chosen. Dilution with ethyl ether and chloroform resulted in curves similar to those with petroleum ether; only a slight difference was noted in the lower relative transmission values of the oils diluted in chloroform. This lowering of the transmission values is believed to be associated with the solubility of the "insolubles" in chloroform over that of the petroleum ether. The use of a low-boiling petroleum ether (isopentane) is not practical, since it rapidly precipitates the polymers from the more highly oxidized oils. Dilutions were accomplished with the aid of calibrated pipets.

The most obvious result of dilution is the large shift in the absorption peak. This amounts to as much as 110 mμ in oils diluted up to 5% with petroleum ether. All oils investigated exhibited approximately the same displacement of the absorption peak with dilution. Figure 3 illustrates this effect. The shift in absorption is not dependent on purity, sample 3 being nearly a pure paraffin oil.

It is obvious that Beer's law will not be rigorously followed by a solution showing such a large shift of the absorption peak as is exhibited with heated quenching oils. Figure 4 shows that there is a uniform change upon dilution in the general nature of the curves. The region of the curve on the ultraviolet side of the peak exhibits a steeper slope than the curve on the red side. These properties must be considered before investigating Beer's law.

To study the extent to which Beer's law is followed, the percent composition (by volume) is plotted against the extinction ( $\log 1/T$ ) at the peak absorption and at a constant wave length (500 and 550 mμ). The graph in Figure 5 shows a curved line for extinctions at peak absorption in the case of the three oils plotted. At 550 mμ the curve, though of lower slope, approaches a straight line. When a filter photometer is used, the curve is a modification of those at 500 and 550 mμ for any given oil. From these

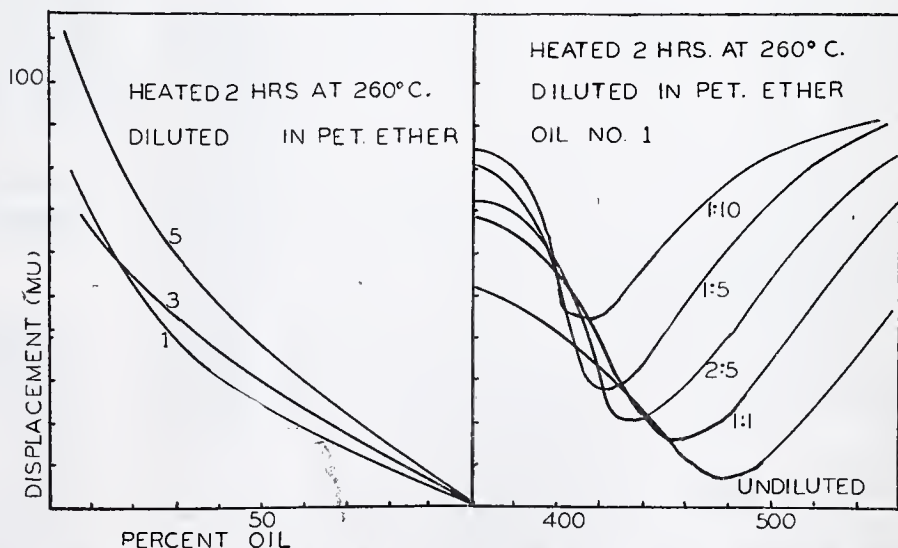


Figure 3. Absorption Peak Displacement vs. Per Cent Composition

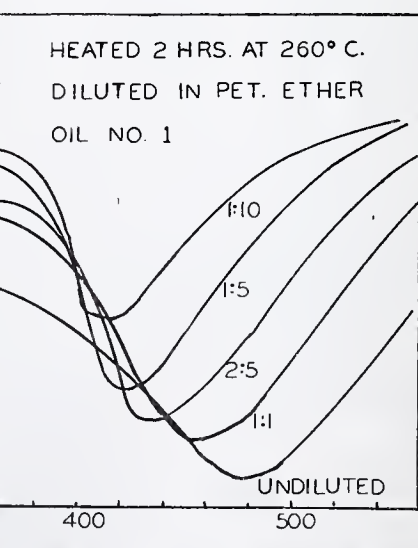


Figure 4. Relative Transmission vs. Wave Length



data it is apparent that Beer's law may be used to correct for dilution only to a rough approximation when the wave length is given.

#### EVALUATION OF OXIDATION STABILITY

Fenske (2, 3) reports the rate of oxidation of lubricating oils to double with each 10° rise in temperature between 140° and 180° C. He has likewise found that the rate of production of volatile and fixed acids, lacquers, and precipitates follows a simple logarithmic function. Small discrepancies with precipitates have been assigned to their "solubility" in isopentane.

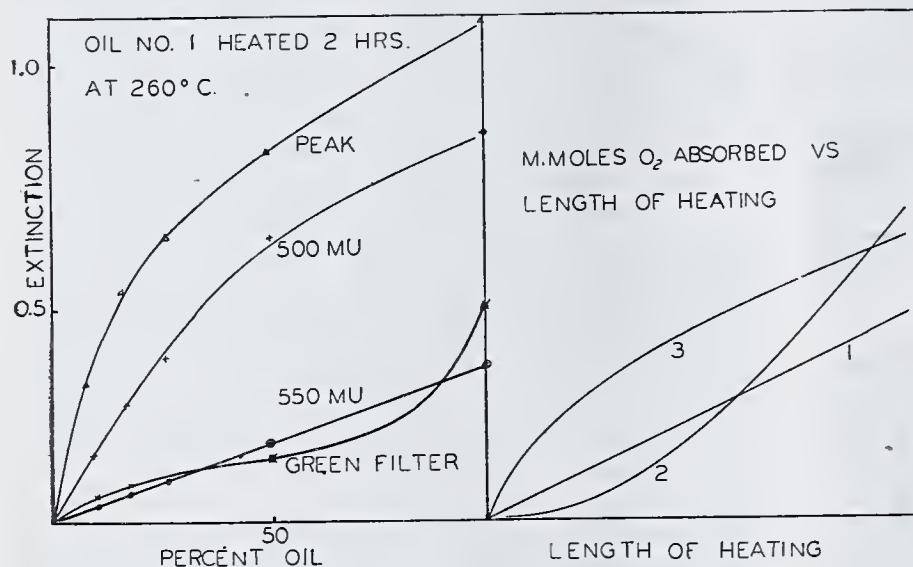


Figure 5. Extinction vs. Per Cent Composition

Three types of curves become evident when the length of heating time is graphed against the amount of oxygen absorbed.

The graph in Figure 6 illustrates this. Curve 1 is characteristic of relatively pure hydrocarbon oils and indicates a uniform rate of oxidation. Curve 2 shows a "period of induction" where the rate of oxidation is slight. After a short time, the oil usually oxidizes at a rate equal to or greater than that of a saturated hydrocarbon. This period of induction may be ascribed to the effect of some natural or added inhibitor. Curve 3 is characteristic of oils heated in the presence of a metallic catalyst, such as the metal of the casting to be quenched. In lubricating oils the metals usually catalyzing oxidation are lead, iron, and copper; in the quenching of aluminum alloys, small percentages of copper may be the predominant catalysts, although many of the authors' experiments indicate that aluminum is not wholly inert (see Table IV).

It is possible to study the relative rates of oxidation of oils by heating a series of oil samples under known conditions of time and temperature and comparing the relative transmission values. In this study the samples were heated in test tubes immersed in an oil bath whose temperature was maintained within 1° of the desired temperature. Only samples from the same series have been compared. The rates of oxidation as shown by this method are not maximum rates of oxidation because of mechanical hindrance of the test tube in permitting the maximum rate of oxygen absorption, but the conditions more exactly parallel those of an actual quenching tank.

The graphs in Figure 7 show the effect of heating various commercial oils at 210° and 260° C. Extinctions are plotted against time of heating, all oils being diluted 1 to 5 with petroleum ether. The slope of such a curve is a measure of the rate of oxidation. In general, the better quenching oils show a period of rapid oxidation followed by a uniform decomposition.

The addition of an antioxidant retards the initial rate of oxidation, but it soon approaches the same rate as the untreated oil. Heating in the presence of a metal catalyst (aluminum alloy turnings) further accelerates initial oxidation in most circum-

stances. If the oil is drastically purified and its rate of oxidation plotted, from the beginning a uniform rate of oxidation is observed. The conclusions to be drawn from these curves are that oils containing aromatic components experience a rapid initial oxidation, while antioxidants inhibit oxidation and catalysts accelerate oxidation. As soon as the less stable components are removed the remainder of the oil (presumably of a more stable naphthenic nature) oxidizes at a uniform rate. Hence, in so far as oxidation stability of quenching oils at a high temperature is concerned, the initial composition (or purity in the sense of more stable hydrocarbons) of the oil is the determining factor. While keeping in mind that maximum rate of absorption of oxygen was not attained in the samples subjected to spectrophotometric tests, it appears that this rate of oxidation with rise in temperature is not so large in the range 200° to 260° C. as it is at lower temperatures except during the short initial oxidation. In these tests the rate of oxidation has doubled in the 50° rise between 210° and 260° C.

#### STUDY OF ADDITIVE ACTION

Most additives used in lubricating oils contain the following essential ingredients: detergents, antioxidants, film strengtheners, and polar compounds designed to improve the lubrication at the surface of the metal. Additives used in quenching oils act primarily to improve oxidation stability and heat conduction.

The function of detergents is to minimize sludge formation and maintain a clean metal surface by inhibiting lacquer formation and buffer acids produced on oxidation (7). Most antioxidants

contain hydroxyl or amino groups or compounds of phosphorus, sulfur, selenium, antimony, arsenic, and germanium. Many theories concerning their mechanism in oils may be found in the literature (2, 3, 4). One important function of some antioxidants lies in poisoning the metallic catalysts. Phosphites have recently been found to increase the proportion of water formed by absorbed oxygen (2, 3), while forming phosphides with suspended metals.

With the aid of the spectrophotometer it is possible to study the antioxidant properties of additives. In general, additives containing antioxidants inhibit the oxidation of the less stable components of the oil. When the antioxidant is added to a highly purified oil the oxidation of the oil is accelerated. This effect may be ascribed to the oxidation of the antioxidant itself. In Table III are compiled the relative transmission values at peak absorption for quenching oil 6 with the additive and its separate components and for sample 6 drastically purified (filtered system oil) by long oxidation and filtration with activated clays. In both oils the halogenated ester has little effect on the oxidation of the oil. The detergent and sulfurized olefin show the greatest

Table III. Relative Transmission Values at Peak Absorption  
(Undiluted, heated at 200° C.)

Sample	Heated 1.5 Hours	Heated 3.5 Hours
System oil filtered	32 (445 mμ)	7 (455 mμ)
S.O. filtered + a <sup>a</sup> 0.3%	27	8.5
S.O. filtered + b 0.06%	31	10
S.O. filtered + c 0.12%	27	8.0
S.O. filtered + d 0.12%	34	11
Oil 6	15 (465 mμ)	3.9 (480 mμ)
Oil 6 + a 0.3%	24	7.7
Oil 6 + b 0.06%	15	4.2
Oil 6 + c 0.12%	22	7.3
Oil 6 + d 0.12%	21	8.0

<sup>a</sup> a. GLC-I composed of b, c, and d.  
b. Chlorinated methyl ester.  
c. Detergent.  
d. Sulfurized olefin.



Table IV. Additive Action

(Heated 3 hours at 210° C. Petroleum ether)

Sample	Transmission Values at Peak Absorption
Oil 6 and lard oil	28 (420 mμ)
Oil 6, lard oil, and Al	31.5
Oil 6, lard oil, and additive I	43
Oil 6, lard oil, additive I, and Al	39
Oil 6, lard oil, and additive II	34
Oil 6, lard oil, additive II, and Al	42

inhibitor action on the untreated oil while producing but slight effect on the filtered oil. Apparently the detergent is the most "active" ingredient in the additive.

Table IV demonstrates the effect of heating the quenching oil with a metal. The aluminum used in this series was in the form of turnings obtained from an aluminum alloy casting. Two types of additives were introduced into samples of the oil: GLC-I, the same as discussed above, and GLC-II containing a phosphite. In all cases the additive has a beneficial effect in inhibiting oxidation. The effect of the aluminum turnings is relatively slight. The increased stability produced by heating oil 5 with aluminum may be attributed to the formation of an aluminum soap with the acids produced by oxidation. The additive GLC-I seems to react with the metal in such a way as to limit its value in inhibiting oxidation, while the second additive is favored by the presence of the aluminum.

CHANGES IN OIL UPON QUENCHING

A series of quenching oil samples from the quenching tanks of a large aluminum foundry was obtained. To the base oil (No. 5) had been added 0.85% lard oil for wetting purposes. Subsequent heating tests indicate little further decomposition of the lard oil over that of the quenching oil when heated at 260° C. To this base oil containing lard oil was added 0.3% of additive GLC-I. If the transmission curves for this composite oil are determined with respect to the oil without the additive, a slightly increased absorption of light is observed which may be ascribed to the color of the additive (Figure 8). However, after the additive has been in the oil for several months the oil actually becomes lighter in color. The effect of decreased light absorption is even more marked in the case of additive GLC-II (freshly added). In this instance, the absorption peak occurs near the absorption peak of the heated oils.

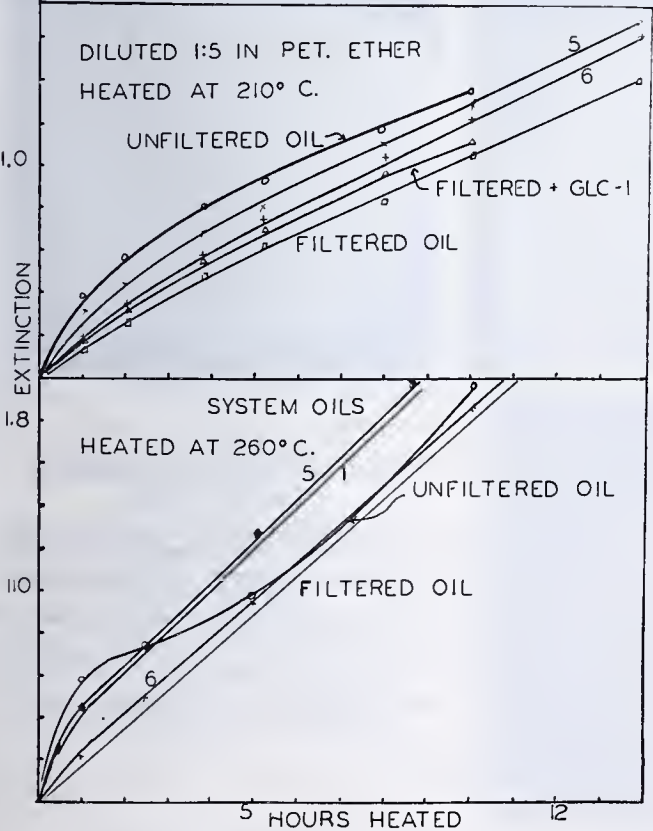


Figure 7. Extinction vs. Length of Heating

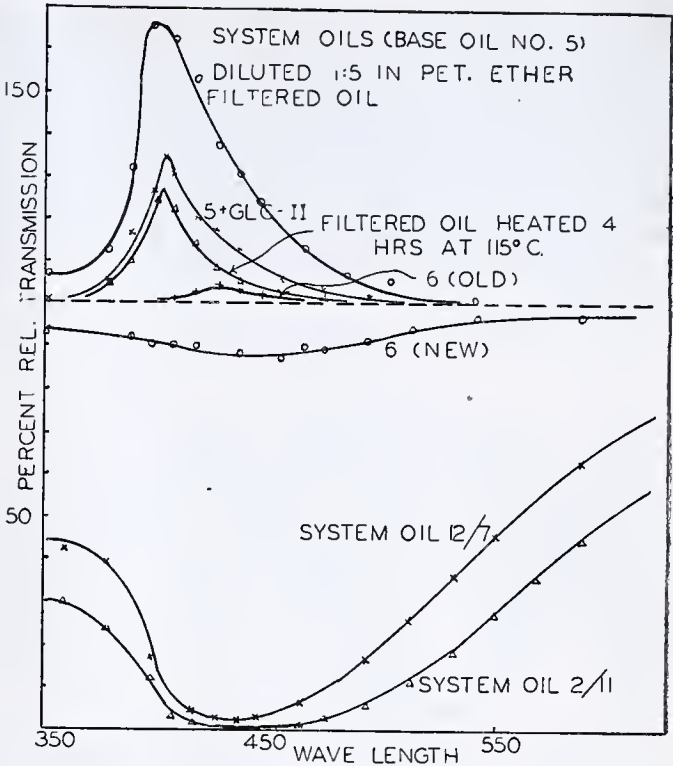


Figure 8. Relative Transmission vs. Wave Length

Table V. Transmission Values of System Oil Relative to Oil Number

(At different dates with continuous use in quenching tank)					
Wave Length, Mμ	12/7	12/18	1/12	2/11 Unfiltered	2/11 Filtered
340	43	40	32	30	112
360	42	37	30	29	115
380	39	32	25	24	117
390	..	..	..	..	137
400	17	16	12	12	170
410	..	..	5	2.2	167
420	3.7	3.1	2.6	1.7	158
430	2.6	2.0	1.8	1.2	142
440	2.3	1.8	1.2	1.0	135
450	2.9	2.0	1.1	1.0	129
460	4.0	2.3	1.3	1.2	..
470	6.0	3.4	2.0	1.8	117
480	8.7	5.0	3.0	2.5	..
500	17	11	7.0	5.2	111
540	36	29	21	18	104
600	63	56	47	44	100

Table VI. Strain in Quenched Castings Compared with Stability of System Oils

(Relative transmission at maximum absorption. Heated at 200° C. Diluted 1 to 5 with petroleum ether)

Sample	Average Strain in Castings, Inches of Bow on Stress Relief		Heated 1 Hour	Heated 2 Hours
	Relief	Stress		
Oil 6	0.055		48 (420)	35 (420)
12/7	0.095		34 (465)	12 (460)
12/18 (first additive)	0.065		42 (470)	19 (475)
1/12 (new additive)	0.045		66 (480)	27 (480)
2/11 unfiltered	0.052		54 (475)	29 (485)
2/11 filtered	0.030		85 (410)	62 (410)
9/11 (6 months' use of oil without further filtering)	0.045		70 (410)	58 (410)

Table V lists the transmission values for samples of quenching oils taken from the quenching tanks at different dates. These samples have not been heated subsequent to removal from the quenching tanks and are relative to the base oil (5) with lard oil and GLC-I (all diluted 1 to 5 with petroleum ether). The transmission values show increasing oxidation over a period of 4 months when the oil was filtered with a clay adsorbent. The filtered oil has a peak transmission 170% greater than the original oil.

If these system oils (from the quenching tanks) are further heated under the laboratory conditions described and the relative transmission curves of the laboratory heated to unheated oils are determined, some information may be had as to the stability of the oil at any time during use in the tank. These values (for peak



relative transmission) are given in Table VI. It might be expected that the stability of the oils should remain constant after the initial heating period (in the quenching tank) but consideration must also be given to the part that new additions of oil, additive, and dissolved aluminum play as the oil is used. The oil filtered after many months of use is observed to be far superior in stability to any new oil. There is a good correlation between these relative transmission values and the strain induced in the aluminum alloy castings quenched in the respective oils.

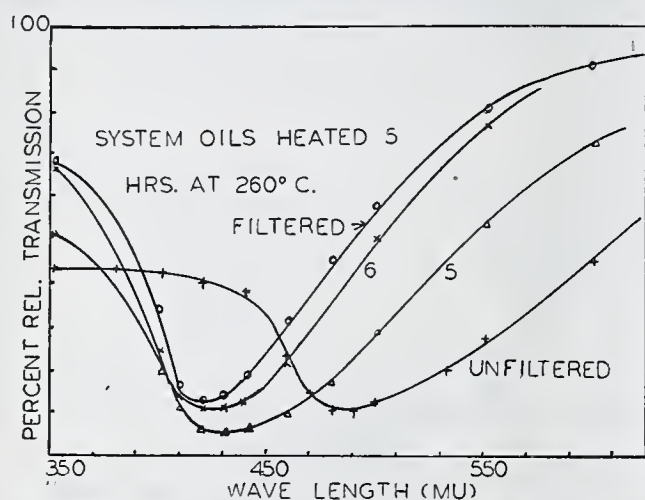


Figure 9. Per Cent Relative Transmission vs. Wave Length

The strain values in Table VI are the amount of bow in inches in the casting during stress relief when the casting is sawed nearly through with a series of parallel saw cuts by a standard procedure for the foundry. Experience in motor performance has shown that a bow less than 0.25 cm. (0.050 inch) has never resulted in failure, but that it is essential to maintain a value less than this maximum. The relation between relative transmission and bow

is not linear in this instance, but this could hardly be expected with an oil which is continually being renewed with fresh oil, two additives of two additive blends, and other variables operating. Without exception, however, in quenching in this 18,925-liter (5000-gallon) tank and in 567-liter (150-gallon) tanks in which single castings are submersed, the lower the relative transmission index number, the higher the residual strain in the castings. It is a curious fact that the variations in strain follow the index numbers in Table VI (relative changes of the oil at any given stage on further oxidation) and not those of Table V (ratio of the transmission of the oil at any stage in the tank to that of the original unused oil).

A further effect is noted when the filtered system oil is heated for 4 hours at 115° C. Under these circumstances the heated oil shows a transmission 126% greater than the unheated filtered oil. Some inhibitor appears to be in this oil, perhaps a stable product from the original additive or from the clay adsorbent. The nature of the transmission vs. wave-length curves for a few of the system oils is given in Figure 9. It is interesting to note the correlation between the absorption curves for samples heated in the laboratory alone and the oils from the quenching tanks.

#### THEORY OF OPTICAL EVALUATION OF OIL DETERIORATION

The above data suggest a theory upon which the observed phenomenon may be explained. Two basic readings may be observed: the natural and relative transmissions which mean natural color and color induced in the oils upon heating. The natural transmission curves are broad and affected to a lesser extent by heating and diluting than the relative transmission curves. The natural transmission curves are the result of colored materials originally present in the oil in addition to any products of oxidation, while the relative transmission curves are characteristic of the heated products of the oil alone. There appear to be three fundamental processes at work in the oil to produce the relative transmission values: absorption, fluores-

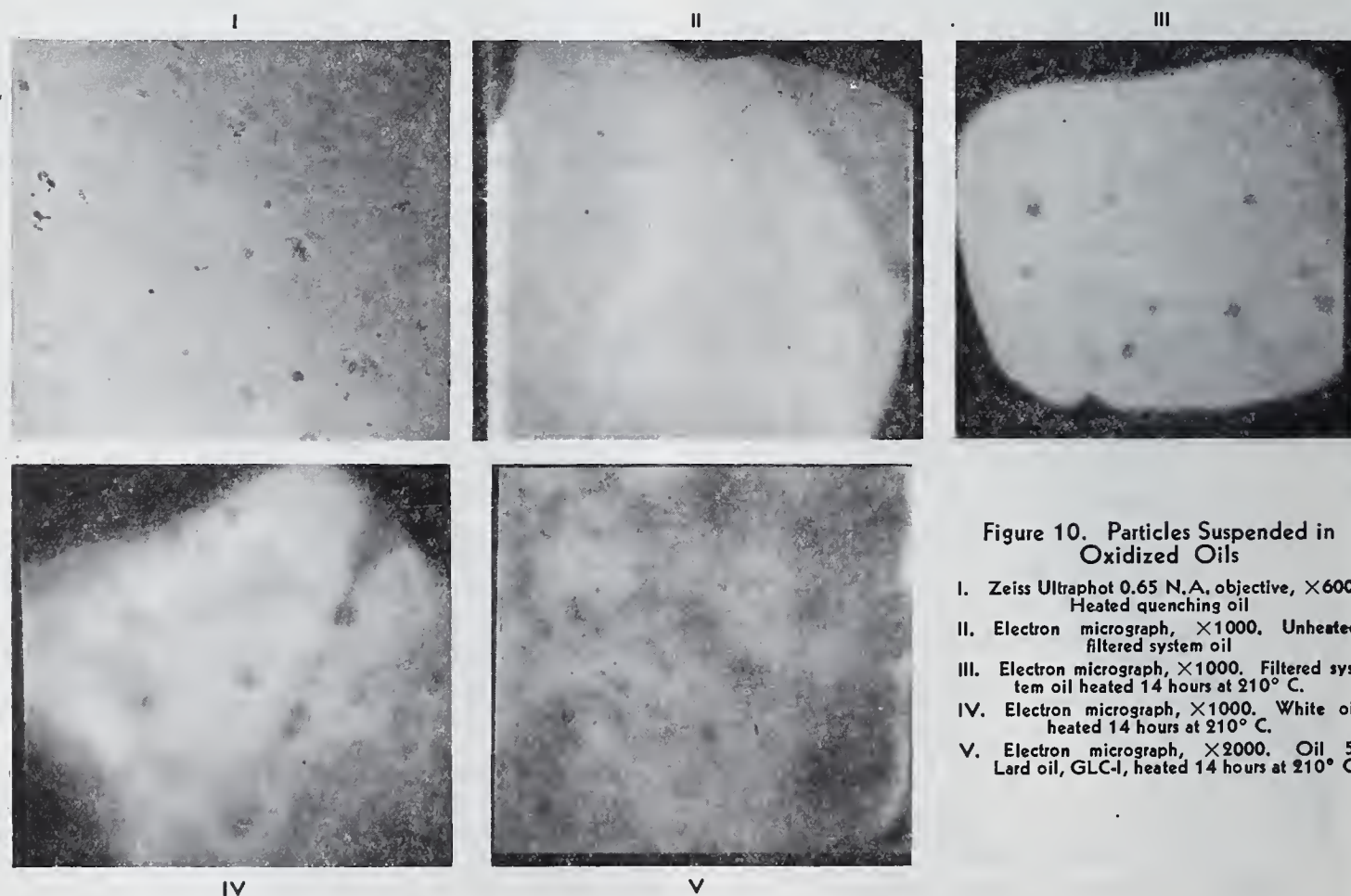


Figure 10. Particles Suspended in Oxidized Oils

- I. Zeiss Ultraphot 0.65 N.A. objective,  $\times 600$ . Heated quenching oil
- II. Electron micrograph,  $\times 1000$ . Unheated filtered system oil
- III. Electron micrograph,  $\times 1000$ . Filtered system oil heated 14 hours at 210° C.
- IV. Electron micrograph,  $\times 1000$ . White oil heated 14 hours at 210° C.
- V. Electron micrograph,  $\times 2000$ . Oil 5. Lard oil, GLC-I, heated 14 hours at 210° C.



ence, and scattering. To what extent each of these processes enters has not been determined, though scattering is believed to play a large part.

The large shift in the absorption peak on dilution with little variation with different solvents does not seem to find adequate explanation on the basis of absorption, while the nature of the curve does not seem to favor explanation on the basis of fluorescence. If absorption were due to fluorescence the transmission curve would be expected to change sharply on the long wavelength side rather than on the short wave-length side of the absorption peak.

The remaining explanation lies in scattering of the light. The presence of finely divided particles suspended in oxidized oils has been known for some time. The "precipitables" are probably a class of these particles. The word "class" is used, since the precipitables can be only the larger of the particles.

Figure 10 illustrates the type of particles to be found in the oil. Photograph I was taken of an undiluted heated oil sample at 600 magnification with a Zeiss 0.65 N.A. objective lens in a Zeiss Itraphot. The particles appear spherical with a tendency to coalesce. Measurement of the size of these particles leads to approximately  $0.5\mu$ . II to V are electron micrographs at 1000 to 2000 diameters. The specimens were diluted approximately 1 to 10 with a benzene drop placed on a collodion film backed with a 100-mesh copper screen and the excess oil removed. It is of interest that any particles appear, since they might be expected to dissolve in the benzene if they are the precipitables.

If these relative transmission curves are actually a measurement of the amount of particles in the oil, the results may indicate the mechanism by which the conduction properties of a quenching oil change on use. The presence or growth of large quantities of particles may have a large influence on the heat conduction from hot casting. This is indicated by the fact that when particles are completely removed by filtration as proved by electron micrographs, the residual strain in castings quenched in this oil as

measured by the bow when the castings are sawed nearly through vertically is decidedly lower than that found in castings quenched in the same oil before filtration. Furthermore, after this filtration following months of deterioration of the oil, remarkable stability is maintained, since in 6 months of continuous use the average strain in castings has increased only from 0.45 to 0.1125 cm. (0.030 to 0.045 inch) and the relative transmission value has decreased from 85 to 70. There is clear evidence from small-scale experiments that with each filtration the stability of this particular oil is still further improved, so that only a very occasional batch operation is required. This may not apply, of course, to other types of quenching oils.

#### ACKNOWLEDGMENTS

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## Determination of Wax in Cotton Fiber A New Alcohol Extraction Method

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A new technique is described for the determination of the wax of cotton fiber. It consists of a two-step process in which the wax is first extracted with hot 95% ethyl alcohol and then transferred to chloroform through a phase-separation process, in order to eliminate tars, mineral constituents, and other nonwaxy constituents removed at the same time by the alcohol. The wax is extracted from cotton fiber more rapidly by hot alcohol than by chloroform, the most rapid and adequate of a considerable number of common wax solvents previously studied. The wax thus determined contains a negligible amount of mineral impurities and is less contaminated with sugars than that determined in the usual Soxhlet extraction.

It is often desirable to know the wax content of different varieties and strains of cotton, as well as of various cotton products which have been subjected to kiering, scouring, or other treatments.

Methods previously available for the determination of wax in cotton are not entirely satisfactory.

A method worked out by Clifford, Higginbotham, and Fargher (3) and based on the study of a number of solvents recom-

mends extraction of 100 grams of cotton with chloroform "in a hot Soxhlet apparatus" for a period of 6 hours; "where it is necessary or desirable, however", the quantity can be reduced to 20 grams and the extraction period to only 3 hours. The use of chloroform, especially "hot", is somewhat objectionable, and the quantities of cotton prescribed are large, often not available, and also beyond the capacities of most conventional Soxhlet extractors. These investigators also recommend that carbon tetrachloride be employed "to give an approximate estimate of wax in cotton, the conditions of time and quantity of material being those already outlined in the case of chloroform". Carbon tetrachloride is rather selective in its action and thus gives a less complete extraction of the wax. These investigators studied a number of other solvents, although not ethyl alcohol.

Ahmad and Sen (1) employed a sample of only 2.5 grams of cotton and extracted for 4 hours. They used hot benzene which, according to Clifford, Higginbotham, and Fargher, extracts more completely than carbon tetrachloride, but less completely than chloroform. It is inflammable, in contrast to carbon tetrachloride.

In addition to chloroform, benzene, and carbon tetrachloride in the cold, Clifford, Higginbotham, and Fargher studied the selectivity and solvent extraction rate of hot and cold petroleum ethers (boiling point  $40^{\circ}$  to  $60^{\circ}$  and  $60^{\circ}$  to  $70^{\circ}$  C.), hot and cold ethyl ether, hot benzene, and hot chloroform. In referring to ethyl alcohol, they stated that it is known to dissolve substances other than wax from cotton. Their results showed that hot extraction, in general, removed in 30 hours or longer more waxy substance than cold extraction and that, of the solvents used, chloroform, benzene, and carbon tetrachloride, in the order

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named, were decreasingly efficient. In all cases the extraction seemed to take place in two stages. During the first stage the extraction is rapid; during the second it is slow, proceeds at a diminishing rate for a long period of time, and appears to be concerned with a different kind of material, difficultly soluble in the solvent. The authors concluded, therefore, that routine measurements with different solvents merely permitted the extractive matter to be divided into classes of varying solubility.

suggested a new technique employing alcoholic extraction, for the determination of wax in cotton fiber. In this the waxy materials are first extracted by the more rapid and inclusive solvent 95% ethyl alcohol, and then separated with the aid of chloroform from the nonwaxy substances. The successful use of this method over a period of some four years on hundreds of samples of raw cotton has seemed to justify its publication.

#### COMPARATIVE EXTRACTIVE EFFICIENCIES OF CHLOROFORM AND ETHYL ALCOHOL

It is appropriate first to establish the advantage of ethyl alcohol over chloroform as a solvent for cotton wax.

Two cottons, one of low and the other of very high wax content, were chosen. For high wax content a 5-gram sample of green lint cotton, studied by Conrad and Neely (5) and having a wax content of about 13%, was employed. For the low-wax cotton a 10-gram sample of ordinary white cotton was used. In each case duplicate samples were weighed out and placed in the extractive compartments of "large" (50 × 250 mm.) Soxhlet extractors. To one set of extractors were added 250 ml. each of chloroform (U.S.P. XI, for anesthesia) while to the others was added an equal volume of 95% ethyl alcohol. Heat was applied to the flasks and the time was noted when the solvent began to condense and fall on the sample; the extractions were continued exactly 2 hours. The extractions were then interrupted, the flasks with extracts set aside, and new flasks with new solvent substituted. The extraction was again continued exactly 2 hours. Both sets of extractions were interrupted in the same way twice more at 1-hour intervals.

In the light of the work of Clifford, Higginbotham, and Fargher (3), the total material extracted by the chloroform may be considered to be wax. However, the waxy material in the alcoholic extract had to be separated from sugars and other nonwax constituents, removed at the same time, by mixing with chloroform and separating into two layers with water, in a manner described below. The accumulated percentages of wax, obtained at the end of the successive periods, are shown by curves in Figure 1.

By reference to Figure 1 it will be seen that not only did 95% ethyl alcohol extract the wax more rapidly than did chloroform but at the end of 6 hours it had extracted a larger quantity. The total wax extracted in 6 hours with alcohol and separated with chloroform was 15% greater than that extracted with chloroform alone from Sample 109CX, and 75% greater than that extracted with chloroform alone from Sample 3120CX. After 6 hours alcohol had extracted as much as or more wax than was obtained with a 6-hour extraction with chloroform.

From Figure 1 it would appear that extraction is not entirely complete after 6 hours even with alcohol. In the case of routine work complete extraction is often not practicable.

For example, although Clifford, Higginbotham, and Fargher (3) actually recovered small but measurable quantities of wax after 35 hours, they recommended only 3 hours for the extraction of a 20-gram sample of cotton with hot chloroform. In the case of an Egyptian cotton extracted with hot chloroform they obtained 0.61% wax after 4 hours, but 0.72% at the end of 5 hours. On the other hand, using cold chloroform on the same cotton they obtained only 0.57% wax after 4 hours and 0.65% after 32 hours. On an American cotton cold chloroform gave them under the same conditions 0.60 and 0.63% wax, respectively. Ordinarily, an extraction period extending beyond about 6 hours is impracticable from a routine standpoint.

The sizes of samples used for the experimental results shown in Figure 1 were one half and one fourth as large, respectively, as the smallest recommended by Clifford, Higginbotham, and Fargher (3) and the extraction was continued for 6 hours instead of 3. For the low-wax cotton the percentage increase in total wax, obtained at the end of the 5th and 6th hours, as compared with that at the end of the preceding hour, was approximately the same for either alcohol or chloroform and less than 3.5%. With the high-wax cotton on the other hand, whereas alcohol extracted 2.14 and 1.58% additional wax during the 5th and 6th hours, respectively, chloroform extracted 10.5 and 8.1%, respectively. In view of the greater total quantity of wax removed, especially in the case

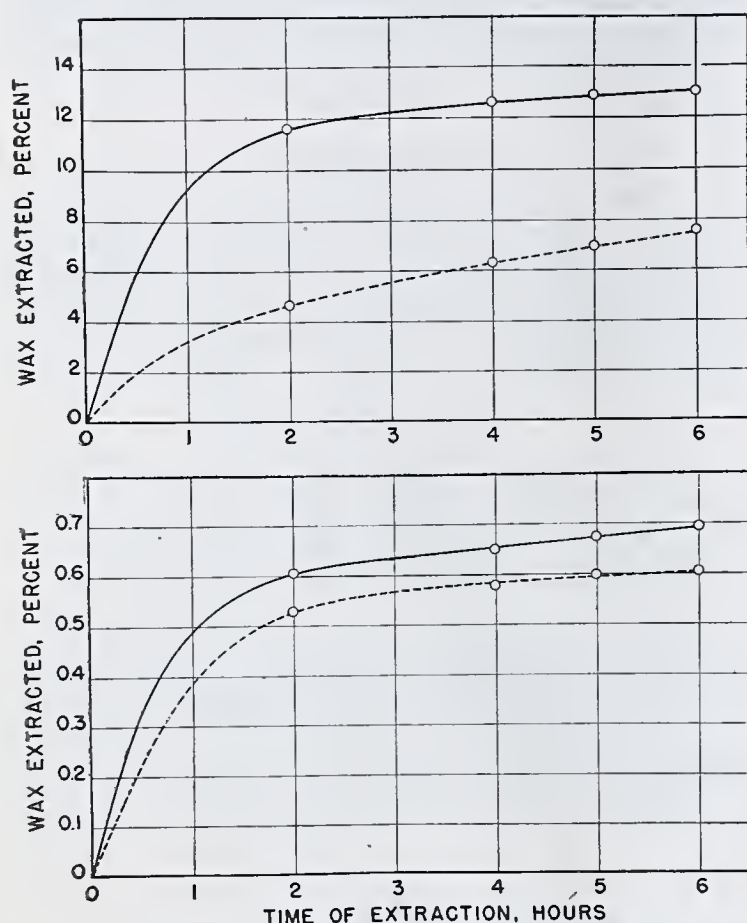


Figure 1. Comparative Amounts of Cotton Wax Extracted with 95% Ethyl Alcohol (Full Lines) and with Chloroform (Broken Lines)

Upper. Very high-wax (green lint) cotton, Sample 3120CX  
Lower. Cotton of normal wax content, Sample 109CX

The heterogeneity of cotton wax from representative samples of American and Egyptian cottons was demonstrated by the rather extensive studies of Fargher and Probert (7), Clifford and Probert (4), and Fargher and Higginbotham (6). These workers found that the crude wax contains such widely diverse classes as long-chain aliphatic mono-, di-, and trihydroxy alcohols, aliphatic acids and esters, sterols, sterol glucosides, a mixture of hydrocarbons, resin acids, and resenes. It is therefore little wonder that the attempt to extract these substances simultaneously with a single "fat solvent" should be beset with considerable difficulty.

Hess (8, p. 205) points out that, in general, the maximum amounts of resins, fats, and waxy substances are extracted from cellulosic materials with acetone or hot alcohol. He credits Schunk (8, p. 206) with the statement that cotton wax is easily soluble in ether and hot alcohol and on cooling separates from the latter in the form of a white gel consisting of microscopically fine crystals. He gives Schwalbe credit (8, p. 249) for the statement that equal parts of alcohol and benzene are appropriate for the quantitative determination of resin, fats, and waxes in cellulosic materials. It will be noted that these authors prefer somewhat different solvents for the removal of waxes from these materials than those recommended by Clifford, Higginbotham, and Fargher (3) for their quantitative determination.

Maclean and Maclean (9, p. 67) point to the fact that alcohol often removes with ease fats and other lipids that are removed only in part or not at all by the usual fat solvents. Bloor (2, p. 264) considers that boiling alcohol gives the most complete extraction of lipids from tissues of any solvent.

A technique used by Thor and Smith (11), in which chloroform was employed to remove the fat and wax from the alcoholic extracts of pecan fruits before undertaking sugar analysis, first



of the high-wax cotton, advantage of alcohol over chloroform—the most adequate solvent of those studied by Clifford, Higginbotham, and Fargher—is clearly demonstrated.

#### TRANSFER OF WAXES FROM ALCOHOL TO CHLOROFORM

The successful use of ethyl alcohol as a wax solvent requires a subsequent treatment to separate the wax from any sugars, amino acids, or other alcohol-soluble, nonwax substances.

Various preliminary experiments were carried out in which the alcohol was evaporated to a very small volume and the resulting mixture washed with water and with various wax solvents, including petroleum ether, ethyl ether, carbon tetrachloride, benzene, and chloroform. Invariably, emulsions were obtained which were unmanageable. Evaporation of the extracts to dryness before use of the wax solvents not only led to the same difficulties, but in addition, the waxy constituents were difficultly and incompletely redissolved.

The preliminary extraction of the cotton with water to remove the nonwax constituents, followed by extraction with alcohol to remove the waxes, proved unsatisfactory, since the water did not wet the cotton readily, often not completely even after 4 hours in the Soxhlet, and channeled through the sample. It was recognized also that hot water might melt some of the waxes and carry them over by entrainment, thus giving low wax results.

The successful procedure, finally adopted, based on the experiments of Thor and Smith (11) and additional suggestions by Thor (10), consisted in the combination of the hot alcoholic extract of the waxes with an equal volume of chloroform, giving a homogeneous solution, and the separation of this into two phases by the addition of water. The waxes are retained by the chloroform layer, whereas the nonwax substances go into the alcohol-water layer.

The completeness with which the wax was transferred to the chloroform layer was demonstrated for both high- and low-wax cottons by separating the first chloroform layer in a separatory funnel, adding new portions of chloroform, and determining the additional amounts of wax obtained. Thus, in a series of samples, using 100 ml. of chloroform for the original separation, and three successive 50-ml. quantities of chloroform to wash the alcohol-water layer, the distribution of wax in the successive portions of chloroform was 99.25, 0.55, 0.10, and 0.10% of the total, respectively. The alcohol-water layers, also, were evaporated to dryness and further examined, both for wax content and for nonwax alcoholic extractives. Any wax obtainable from these layers amounted to less than 0.02%, absolute, while the nonwax residue amounted to 0.75 to 1.30% of the sample, depending on the sample used. It is thus evident that the transfer of waxy substance from alcoholic solution to chloroform is very complete with the first 50-ml. chloroform wash.

#### AMOUNT OF ASH CARRIED TO CHLOROFORM WITH WAX

Experiments were conducted on both low- and high-wax cottons which showed that while the alcoholic extractions removed 37% of the total mineral constituents from the low-wax cotton and 25% from the high-wax cotton, the greatest weight of ash found in the wax was 0.7 mg., and the average was 0.3 mg. These figures are equivalent to a maximum error in the wax content of 0.44% or an average error of only 0.15%. It is thus evident that no appreciable error is introduced into the wax results through transfer of mineral constituents from the alcoholic to the chloroform solution.

#### AMOUNT OF SUGARS CARRIED TO CHLOROFORM WITH WAX

The question also arose as to whether any appreciable quantity of sugars (which are contained in cotton fibers in slight amounts and dissolve in 95% ethyl alcohol) could, because of slight solubility in chloroform, be transferred over into it and thus lead to greater apparent weight of wax than actually exists. Sugars would be about the only other nonmineral, nonwaxy constituents known to be present which are soluble in aqueous alcohol and might be soluble to a slight extent in chloroform. No exactly pertinent data could be found in the literature.

To decide this question duplicate 1-gram samples of dextrose, the principal sugar found in the fiber, as well as like samples of sucrose and levulose were dissolved in 100-ml. quantities of 95% ethyl alcohol, transferred to 100 ml. of chloroform, and washed with three additional successive 50-ml. portions of chloroform in the same way as was done above with the alcoholic extracts of the fibers. The chloroform extracts were evaporated to dryness, first on the steam bath and then in the vacuum oven at 80° C. The residues were weighed and the weights of the sugars found in this way are shown in Table I.

Table I. Sugars Transferred to 250 Ml. of Chloroform

(By distribution from 95% ethyl alcoholic solutions containing 1 gram of the sugar)

Sugar Taken	Residue from Chloroform Mg.	Sugar Recovered Mg.
None	0.9 0.9	...
Dextrose	3.4 3.8	2.5 2.9
Sucrose	1.1 1.2	0.2 0.3
Levulose	1.9 1.8	1.0 0.9

By reference to Table I it will be seen that the chloroform itself contained a very slight residue. Very small quantities of sugar dissolved in the chloroform, the amount being somewhat the greatest in the case of dextrose. Assuming a 10-gram sample of cotton of about average wax content of 0.5% and dextrose as only sugar in the fiber, the error caused by a saturated solution of this sugar in 150 ml. of chloroform (two washes of 100 and 50 ml., respectively) is slightly over 3% of the wax. On the other hand, it is evident that in a conventional extraction where the chloroform would be used directly in the Soxhlet apparatus to remove the wax, and where through repeated siphoning the effective volume of the chloroform is many times that actually present, a much greater quantity of sugars could be dissolved if present. In fact, an actual experiment showed that under these conditions from 10 to 25 times as much sugar could be extracted in a 6-hour extraction period, and deposited in the extraction flask. The use of alcohol for the extraction, with subsequent transfer to chloroform, thus avoids a serious source of error which can be present if chloroform is used directly as the wax solvent.

#### PROPOSED METHOD FOR DETERMINATION OF TOTAL WAX IN COTTON FIBER

The following method is proposed for the determination of total wax in cotton fiber and similar materials.

Place 5 to 10 grams, depending on the wax content, of well cleaned fiber in a coarse thimble in a large Soxhlet (50 × 250 mm.) extractor assembled ready for operation. Add 250 ml. of 95% ethyl alcohol to the extraction flask and adjust the gas flame or other source of heat until the liquid siphons over at 3 to 4 minute intervals. Continue the extraction for 6 hours. Turn off the heat, lift condenser, and remove the thimble and sample from the extraction compartment. Replace condenser and continue heating until part of the alcohol has passed over to the extraction compartment of the Soxhlet and only 75 to 85 ml. of liquid remain in the extraction flask.

While still warm (above 60° C.), or after warming if the extract has been allowed to cool, transfer the alcoholic extract to a 500-ml. separatory funnel. Wash out the Soxhlet flask with several 5-ml. portions of hot 95% ethyl alcohol and add additional alcohol, so that the final volume is approximately 100 ml. Add 100 ml. of reagent or U.S.P. XI grade chloroform to the separatory funnel and mix thoroughly. This should give a completely homogeneous solution. Now add to this alcohol-chloroform solution 75 ml. of water and agitate somewhat to cause mixing and separation of two distinct layers, the chloroform layer being at the bottom. Do not agitate violently, as this is unnecessary and tends to cause permanent emulsions to form in some cases. Allow the two layers to stand until they become clear. This may take overnight. Draw off the chloroform layer and set aside in a 250- to 300-ml. Erlenmeyer flask. Add a fresh 50-ml. portion of chloroform to the separatory funnel,



agitate gently, and again allow the layers to separate. This time the separation should be complete in a couple of hours. The wax is now practically completely in the chloroform layer.

It is probably desirable to wash the chloroform solution of extracted wax at least once with water. Therefore drain the separatory funnel and discard the spent alcohol-water solution, which also contains the sugars and other alcohol-soluble, non-waxy substances. Without washing the funnel, pour the chloroform solution of wax back; add about 100 ml. of distilled water, shake carefully, and allow the two layers to separate. When separation is complete, draw off the chloroform layer, receiving it into the same Erlenmeyer flask from whence it was last taken. Now add two 5-ml. portions of fresh chloroform successively to the separatory funnel, shake well, allow to separate, and draw off each in turn into the Erlenmeyer flask containing the main body of chloroform solution. This should complete the transfer of the wax back into this flask.

Remove the chloroform from the wax by evaporating in tared 100-ml. beakers on a steam bath. The beakers should not be filled more than half way at a time, because of the tendency for the chloroform to superheat and boil up slightly at times as well as to leave a deposit of wax above the solvent on the sides of the beaker. After the wax residue appears to be dry, cool and weigh the beakers, then heat them on the steam bath 30 minutes more, and again cool and weigh. If the weights are not constant, repeat reheating until two successive weighings agree within 0.1% of the residue weight.

#### ACKNOWLEDGMENT

The assistance of Meyer D. Silverman and James H. Kettering in obtaining the data herein reported is gratefully acknowledged.

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## A Fractionating Column

### For Continuous Production of Distilled Water of High Purity

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THE entire apparatus shown in Figures 1 and 2, the fractionating column of which is of primary interest here, was used to prepare redistilled water of high purity from a laboratory supply of rather poor quality. Long single tubes of small inside diameter and concentric glass tubes, in which the reflux is distributed on the walls of the tubes have been found to give an efficiency of up to the equivalent of more than 85 theoretical plates in a length of less than 150 cm. (5 feet) (2). The present apparatus may be regarded as two such columns in series, but arranged concentrically to prevent loss of heat and to reduce the over-all height.

Accessories shown serve to make the operation of the apparatus continuous and automatic. The constant-level device for controlling the input is a more sturdy modification of one previously reported (1). To maintain an easily controlled uniform flow of cooling water through the condenser, an obvious device was used (constant head, Figure 2).

Present circumstances do not justify a careful study of the heat exchange and other details of performance of the apparatus. Instead, certain seemingly valid assumptions were made, and the final effectiveness of the column was tested by a simple electrometric measurement of the conductivity of the distillate (water).

It is assumed that the greater part of any higher-boiling fractions are condensed in the outer space between the outer air-cooled shell and the middle concentric tube and are refluxed back into the boiling flask, and that, at least in the case of water, any heavier fractions which pass over the top in more than negligible traces are substances that will readily distill with steam and will be carried with the steam to the top of the condenser. The amount and composition of the first reflux (above) will, of course, depend on temperature and rate of flow of the vapors, temperature of the surrounding air, and other factors. The narrow space between the central and middle concentric tubes serves to conduct the remaining vapors to the bottom of the central space and to maintain or slightly increase their temperature toward the bottom of the space. These vapors then ascend

through the central space to the condenser, which is maintained by slow flow of cooling water at near the temperature of condensation of water. As the condensate flows down the walls of the central tube, it is continually in contact with hot vapor flowing upward. Any lighter fractions which may have condensed and any gases redissolved in the water are assumed to be driven off again and returned to the top of the column. Gases and lower-boiling fractions are eventually driven out with a small portion of steam from the top of the condenser.

On several occasions the performance of the still was tested by measuring the conductivity of the distillate in a Barnstead purity meter in terms of an electrometrically equivalent concentration of sodium chloride in solution in pure water. The results are given in Table I. From these data it is evident that the first 150 to 200 ml. should be discarded, that the still is then clean and

Table I. Conductivity of Distillate (Water) Delivered at 3 to 4 Liters Per Day

Run	Sample, Portion of Run, Condition of Still, etc.	Temp. ° F.	NaCl Equivalent P.p.m.
1	Water in reservoir	80	5.8
	First distillate taken, read when taken	130	0.3
	First distillate taken, after 30 min.	105	0.5
	Probable solution of electrolyte from glass		
2	Beginning of day, first 150 ml. discarded	95	<0.1
	First sample, barely in zero range	132	<0.1
	End of day, far into zero range		
3	End of 2-liter run, last portion from reservoir in flask, far in zero range	132	<0.1
4	Still idle 2 weeks; first 20 ml. discarded	128	0.7
	First fraction, 50 ml. after 20 ml. discarded		
	40 ml. discarded, next 50 ml. taken }	130	<0.1
	Second fraction, well in zero range }		

A considerable arc on the meter below 0.1 is marked "zero range".



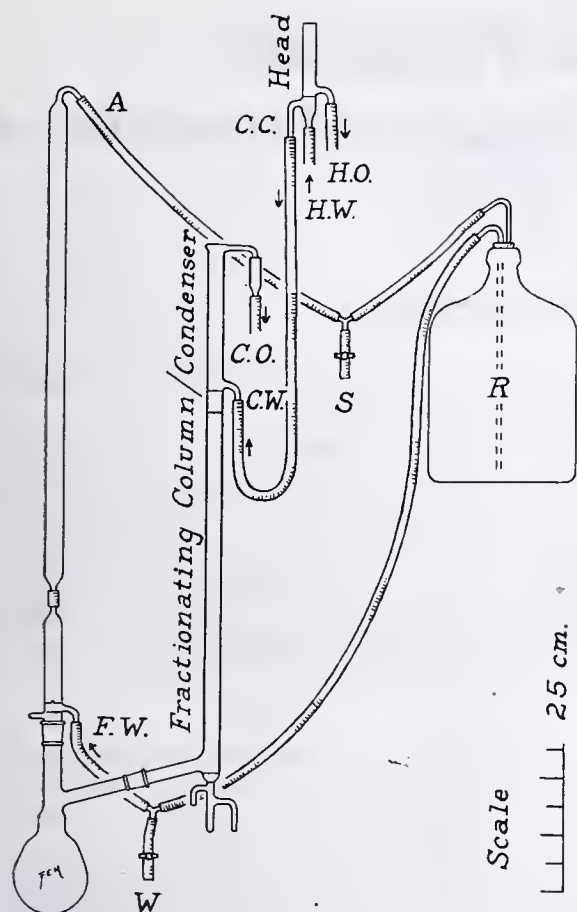


Figure 1. General Arrangement and Proportions of Column, Boiling Flask, and Accessories

- A. Air pressure communication, constant-level column to R
- C.C. Condenser cooling water control capillary
- C.O. Condenser cooling water overflow
- C.W. Condenser cooling water inlet
- F.W. Feed water (from R into boiling flask)
- Head. Constant head device
- H.W. Constant head device, inlet (tap water)
- H.O. Constant head device, overflow
- R. 20-liter bottle, reservoir for feed water
- S. Tube with screwclamp for application of suction
- W. Tube for refilling R with water

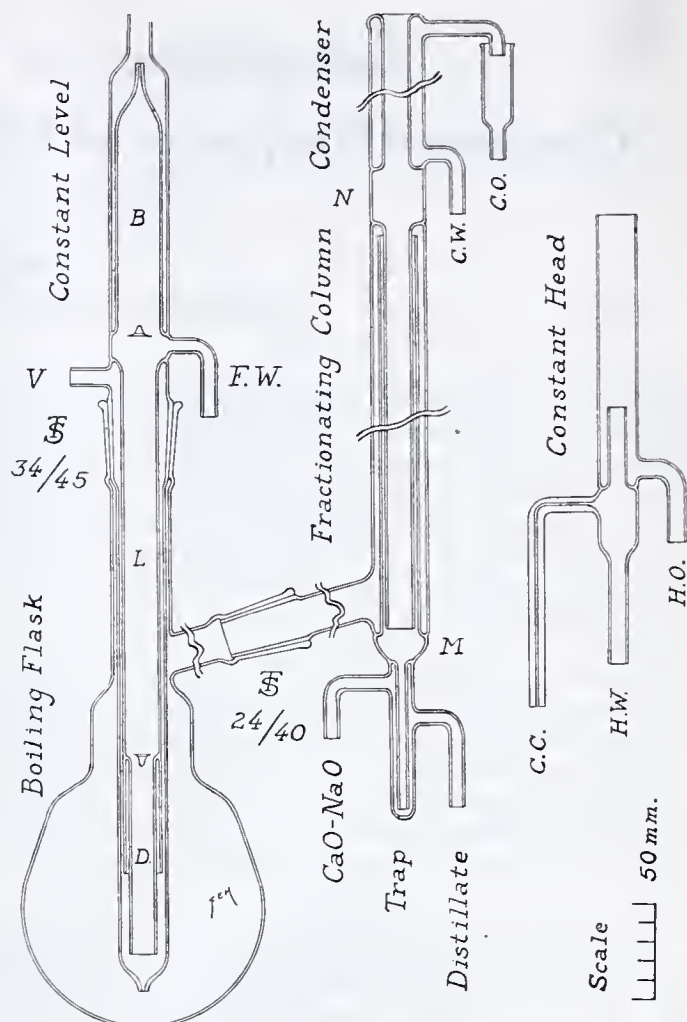


Figure 2. Details of Column, Boiling Flask, and Accessories

- B. Baffle
- D. Tube to check air flow and facilitate surge of water
- L. Constant-level tube
- M. Dead space below central tube
- N. Space separating condenser from top of fractionating column
- V. Vent
- Other reference letters same as in Figure 1
- § Standard-taper joints as indicated

delivering pure water, and that the production of a satisfactory distillate is maintained to the last portion of at least 20 liters from the reservoir.

Space N, Figure 2, separates the condenser from the fractionating column to prevent overcooling of the first reflux. Space M, which is undesirable and is made as small as possible, could be heated slightly to prevent condensation of lighter fractions, but for present purposes this has not been found necessary. The trap serves to prevent loss of vapor and to maintain sufficient back pressure to force the vapor through the column. If extreme precautions are to be taken, a soda-lime tube or other protective absorbent may be connected to the trap vent (CaO-NaO, Figure 2).

A uniform flow of cooling water is essential to proper operation of the still. This is adequately provided by a constant head or pressure of water acting against the resistance of the capillary (Figure 2, C.C.) and by the fixed level of the overflow from the condenser into an open cup (C.O. in both figures). The device marked "constant head" in Figure 2 is suitable. Its position above the column is indicated in Figure 1. By raising or lowering it, the rate of distillation and the portion of middle fraction rejected, hence also the quality of the distillate may be closely controlled.

The constant boiler level control device consists of two parts, built separately for convenience in fabrication and to decrease danger of breakage. Air entering the lower section through the vent, V, passes below the bottom edge of L, upward between the walls of L and D, through the V-shaped opening in the ring seal into L and then B, and through the capillary tip of B into the top section of this column. Water from B and L surges freely down D when displaced by air, quickly blocking further ingress of air, but it can flow only slowly past B through the V-shaped opening in the ring seal at the bottom of B. Air flows from the top of the column at A into R, permitting water to flow from R to F.W. If,

Table II. Dimensions of Still

Fractionating column, length 600 mm.	Condenser, length 300 mm.
Outer tube (shell) 30 mm. O.D.	Outer tube 30 mm. O.D.
Middle tube 20 mm. O.D.	
Central tube 15 mm. O.D.	Central tube 15 mm. O.D.
Shell of lower section of constant-level control above standard taper joint, and entire upper section of column	32 mm. O.D.
Shell of lower section below standard taper joint	25 mm. O.D.
Tube B 24 mm. O.D. Tube L 19 mm. O.D. Tube D 14 mm. O.D.	
Orifice at top of baffle, B, approx. 0.6 mm. I.D.	
Orifice at bottom of shell of lower section, approx. 1.5 mm. I.D.	
Bottom edge of L approx. 30 mm. above desired level in flask	
Trap at least 50 mm. long between lower end of inner tube and junction of distillate delivery tube, because of back pressure	
Control capillary of constant-head device approx. 1.0 mm. I.D. and 150 mm. long including bend	
Constant-head device large enough to avoid flooding and splashing	

in starting operation, the pressure in R is not sufficiently low to hold back excess water, it can be reduced further by applying suction slowly at S. The reservoir can be refilled through W by placing pinchclamps between A and S and between F.W. and W and applying suction at S. The earlier paper (1) gives a more satisfactory explanation of the constant-level control.

Considerable latitude is probably permissible in the dimensions of various parts of the still. Those that may be informative or critical are listed in Table II.

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# Salicylimines as Organic Precipitants

## Quantitative Precipitation of Nickel and Copper and Determination of Copper in Brass or Bronze

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ETTLING (2) in 1840 showed that salicylaldehyde yields an insoluble compound when treated with ammoniacal cupric, nickel, or ferric ions. Schiff (4) showed that the compounds obtained were complexes of the ion with salicylimine, formed by reaction of ammonia with the aldehyde, and that primary amines could replace ammonia in the reaction, giving rise to *N*-alkyl salicylimine complexes. Pfeiffer, Buchholz, and Bauer (3) prepared a number of salicylimine complexes and studied their properties.

The present work deals with the qualitative reactions of metallic ions with salicylaldehyde and its 5-bromo, 5-nitro, and 3,5-dibromo derivatives in ammonia and in methylamine solution. All tests were run in the presence of 5% sodium tartrate to prevent precipitation of the hydrous oxides. The quantitative determination of copper and nickel in aqueous solution, and of copper in brass or bronze is described.

### REAGENTS

Salicylaldehyde, obtained from the Eastman Kodak Company, was redistilled and that portion boiling from 195° to 196° C. was used in the investigation. 5-Bromosalicylaldehyde (m.p. 124° C.), 5-nitrosalicylaldehyde (m.p. 124–125° C.), and 3,5-dibromosalicylaldehyde (m.p. 81–82° C.) were prepared according to Beilstein (1).

IMINE SOLUTIONS. *Salicylimine*. Dissolve 1 gram of salicylaldehyde in 100 ml. of 10 to 90 ammonium hydroxide. *5-Nitrosalicylimine*. Dissolve 1 gram of 5-nitroaldehyde in 100 ml. of 10 to 90 ammonium hydroxide. *5-Bromosalicylimine*. Dissolve 0.25 gram of the 5-bromoaldehyde in 100 ml. of concentrated ammonium hydroxide. *3,5-Dibromosalicylimine*. Dissolve 0.2 gram of the 3,5-dibromoaldehyde in 100 ml. of concentrated ammonium hydroxide. The solutions darken and lose their usefulness after approximately 8 hours.

*N-METHYLIMINE SOLUTIONS*. Prepare in same way as imine solutions, using 25% aqueous methylamine in place of concentrated ammonium hydroxide. The solutions are stable for long periods of time.

*SOLUTIONS OF INORGANIC IONS*. Stock solutions of known approximate concentration were prepared from reagent grade salts, oxides, or metals, followed in some cases by reduction on amal-

gamated zinc. The solutions were diluted to contain 1 to 2 mg per ml. for making the tests.

### PROCEDURE

To 5 ml. of 5% sodium tartrate solution containing 0.5 to 1 mg. per ml. of the ion were added 2 ml. of the reagent. The solution was examined for the presence of a precipitate or color. The sensitivity of the tests was determined by adding 2 ml. of the reagent to 10 ml. of the ion in appropriate concentration. After allowing 10 minutes for reaction the tubes were examined for a Tyndall cone in a narrow beam of light. In each case a blank was run on distilled water for comparison.

### RESULTS

All the metallic ions obtainable in aqueous solution were tested with the exception of the following: all ions of Rb, Cs, Ra, Sc, Ac, Zr, Hf, Cb, La, Pa, Ma, Rh, Ir, Tl, Ge, and Po. The results are summarized in Tables I and II.

EFFECT OF pH. In all cases the imines are unstable in even weakly acidic solutions. The precipitates were insoluble in high concentrations of the amine or ammonia. Thus, the insolubility range in all cases is pH 7–8 to 11–12.

### QUANTITATIVE APPLICATION OF SALICYLIMINE

Standard solutions of  $\text{Cu}^{++}$  and  $\text{Ni}^{++}$  were prepared by electrolyzing a known amount of the metal on platinum, removing the metal plate with nitric acid, and diluting to known volume. To pipetted samples of the standard solutions were added sodium tartrate to 5%, followed by an excess of ammonia and salicylimine solution prepared according to the above directions. The precipitates were filtered through sintered-glass crucibles and weighed after being dried to constant weight at 100° C. The factor for converting the copper precipitate to metal is 0.2092, and the factor for nickel is 0.1963.

Cu Taken, Mg.	Wt. of Ppt., Mg.	Cu Found, Mg.
9.85	46.9	9.81
9.85	47.1	9.85
24.87	118.6	24.81
24.87	118.6	24.81
49.58	236.9	49.56
49.58	236.6	49.50

Table I. Reactions of Salicylaldehyde and Derivatives

Ion	Salicylaldehyde		5-Nitro		5-Bromo		3,5-Dibromo	
	$\text{NH}_3$	$\text{CH}_3\text{NH}_2$	$\text{NH}_3$	$\text{CH}_3\text{NH}_2$	$\text{NH}_3$	$\text{CH}_3\text{NH}_2$	$\text{NH}_3$	$\text{CH}_3\text{NH}_2$
$\text{Cu}^{++}$	Green ppt.	Green ppt.	Green ppt.	Green ppt.	Green ppt.	Green ppt.	Green ppt.	Green ppt.
$\text{Ni}^{++}$	Orange ppt.	Yellow ppt.	Orange ppt.	Yellow ppt.	Yellow ppt.	Yellow ppt.	Brown ppt.	Yellow ppt.
$\text{V}^{++}$	Red ppt.	Red ppt.	Orange ppt.	Red ppt.	Red ppt.	Red ppt.	Red ppt.	Red ppt.
$\text{Pd}^{++}$	Yellow ppt.	Yellow ppt.	Yellow ppt.	Yellow ppt.	Yellow ppt.	Yellow ppt.	Yellow ppt.	Yellow ppt.
$\text{Mn}^{++}$	....	....	....	....	....	....	....	Brown ppt.
$\text{Fe}^{+++}$	Red ppt.	Red ppt.	....	....	Red ppt.	....	....	....
$\text{Fe}^{++}$	Red color	Red ppt.	Red color	Blue ppt.	....	Lavender ppt.	....	Purple ppt.
$\text{Co}^{++}$	....	Brown ppt.	Brown ppt.	Brown ppt.	....	Brown ppt.	Brown ppt.	Brown ppt.
$\text{Hg}^{++}$	....	....	....	....	....	....	....	Yellow ppt.
$\text{Zn}^{++}$	....	....	....	....	....	....	....	Yellow ppt.
$\text{Cd}^{++}$	....	....	....	....	....	....	....	Yellow ppt.
$\text{ReO}_4^-$	....	....	....	....	....	....	....	Yellow ppt.

Qualitative reactions in 5% sodium tartrate. .... indicates no reaction.

Ni Taken, Mg.	Wt. of Ppt., Mg.	Ni Found, Mg.
8.91	44.9	8.81
8.91	45.1	8.85
22.57	115.4	22.65
22.57	115.2	22.61
44.78	227.6	44.68
44.78	228.0	44.76

### DETERMINATION OF COPPER IN BRASS OR BRONZE.

A 60- to 100-mg. accurately weighed sample of the alloy is dissolved in hydrochloric-nitric acid (2 ml. of each of the concentrated acids). The solution is diluted to approximately 25 ml. and sufficient sodium tartrate solution is added to make its over-all concentration 5%. After making distinctly ammoniacal with filtered ammonium hydroxide and cooling below 25° C., the salicylimine solution is added. After 5 minutes in the cold the precipitate is filtered through sintered-glass crucibles, dried for an hour at 100° C. (never above 105° C.), and weighed. Per cent copper is calculated as follows: % Cu = weight of ppt.  $\times$  0.2092  $\times$  100/sample



weight. The procedure was applied to Bureau of Standards brass 37B and bronze 52 samples, with the following results:

Brass, Mg.	Wt. of Ppt., Mg.	% Cu	Bronze <sup>a</sup> , Mg.	Wt. of Ppt., Mg.	% Cu
124.9	421.3	70.56	99.0	418.8	88.50
73.2	249.5	71.30	64.8	273.7	88.75
79.6	267.7	70.36	90.0	381.0	88.55
96.0	323.0	70.38	107.7	455.2	88.42
90.2	303.2	70.32			
67.0	225.9	70.53			
71.5	241.2	70.57			
89.0	300.3	70.58			
Bur. of Standards average		70.36			88.33

<sup>a</sup> Contains 0.13% Ni, which was not subtracted.

**INTERFERENCES.** The interferences may be found by reference to Table I. Less than approximately 0.3% of iron does not interfere.

## Determination of *o*-Xylene in Recycle Styrene

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A procedure for determining *o*-xylene in the presence of alkylbenzenes and olefinic compounds consists of removing the unsaturates with mercuric acetate, nitrating the *o*-xylene and other alkylbenzenes, and, using a modified Bost-Nicholson reaction, measuring the color produced by dinitro-*o*-xylene in a suitable photoelectric colorimeter. It is possible to detect less than 0.01% *o*-xylene in samples consisting largely of unsaturates.

IN THE production of Buna S rubber the recovery of unpolymerized styrene monomer is an important factor. Impurities such as 1,4-vinylcyclohexene, ethylbenzene, isopropylbenzene, *n*-propylbenzene, and *o*-xylene accumulate in varying amounts, thus making purification necessary to utilize the unpolymerized styrene. All these impurities except *o*-xylene, which boils at 144° C. (styrene boils at 145–146° C.), are easily removed by distillation. Therefore the analysis of styrene for *o*-xylene is often important.

Luszczak (4) developed a method for the determination of small amounts of xylene in xylene-toluene vapor mixtures in the air within buildings, in which 7 to 8 liters of air were shaken for half an hour in a flask with 50 to 100 ml. of alcohol. The xylenes, which give the same millimolar extinction constant, and toluene, whose extinction curve is not simultaneously influenced, were quantitatively determined from the ultraviolet spectrum of the alcoholic solution. Luszczak (5) also developed a similar method for the determination of benzene, toluene, and xylene in commercial benzene.

A method for analysis of *m*-xylene in xylene mixtures by nitration and fractional crystallization of 2,4,6-trinitro-*m*-xylene from acetone was described by Reichel (7).

Kester and Holmes (3) analyzed mixtures of paraffins, benzene, toluene, and xylene by means of fractionation followed by sulfonation of the cuts. Zaborowski (12) analyzed various mixtures of xylene, toluene, and gasoline by sulfonation with a known amount of sulfuric acid and titration of the excess acid.

An optical method was developed by Schildwächter (8) in which vapors of methanol, ethanol, diethyl ether, pentane, hexane, heptane, benzene, toluene, xylene, and various petroleum and coal-tar benzenes were determined. The requisite percentages were calculated from data obtained by means of an interferometer.

Norris (6) devised a method for the determination of *o*-xylene in xylene mixtures by oxidation with potassium permanganate to the corresponding phthalic acids, which were separated and determined. However, this method is only about 90% quantitative.

Table II. Sensitivity of Reactions

Ion	Salicylaldehyde		5-Nitro		5-Bromo		3,5-Dibromo	
	NH <sub>2</sub>	CH <sub>3</sub> NH <sub>2</sub>	NH <sub>2</sub>	CH <sub>3</sub> NH <sub>2</sub>	NH <sub>2</sub>	CH <sub>3</sub> NH <sub>2</sub>	NH <sub>2</sub>	CH <sub>3</sub> NH <sub>2</sub>
Cu <sup>++</sup>	2	0.75	3.5	1.5	4	1.5	4	2
Ni <sup>++</sup>	2	0.75	3	1	3	2	3	1.5
CO <sup>++</sup>	.	0.5	1	0.75	1.5	1	2	1.5
Fe <sup>++</sup>	.	0.1	...	0.25	...	0.75	.	1

Sensitivity of tests, 10<sup>6</sup> ml. of solution in which 1 gram of ion can be detected.

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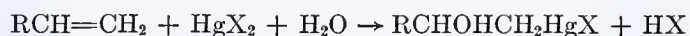
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Although some of these methods are accurate, none seemed suitable for the analysis of small amounts of *o*-xylene in the type of samples under examination. Furthermore, infrared methods (11) for *o*-xylene are not possible in this case because of the interference of ethylbenzene. Therefore it was found necessary to develop an entirely new procedure.

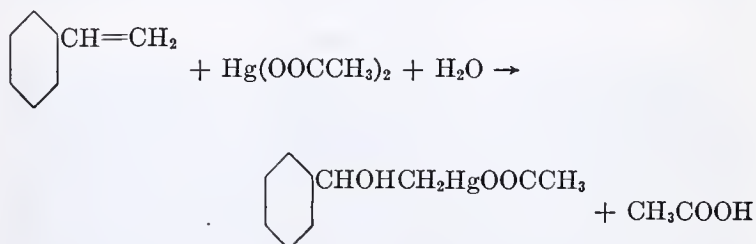
### OUTLINE

I. The alkylbenzenes are separated from the styrene, 1,4-vinylcyclohexene, and any other olefinic compounds present by reacting the sample with aqueous mercuric acetate and subsequently steam-distilling the alkylbenzenes from the reaction mixture. Ethylbenzene is used as a carrier in the steam-distillation to aid in removing the *o*-xylene.

Whitmore (9) states that aqueous mercuric salts add the groups —HgX and —OH to the double bond of olefinic compounds. A general equation for this reaction may be shown as follows:



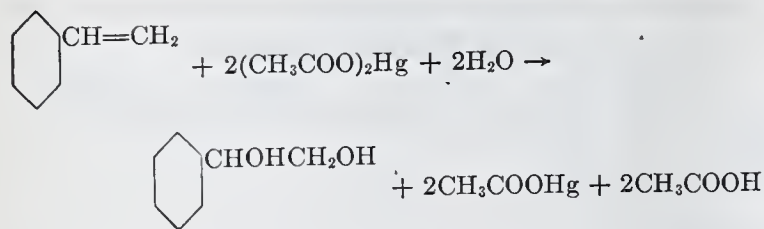
the addition in general following Markownikoff's rule (10), mercury going to the carbon having the most hydrogen atoms. With styrene, the reaction with mercuric acetate would be:



Mercuric acetate reacts similarly with the unsaturated linkages of the 1,4-vinylcyclohexene.

In a few instances, particularly with the propenyl group, (—CH=CHCH<sub>3</sub>), the mercuric salt does not add to the double bond, but instead oxidizes it to the glycol (1). Thus, if this reaction occurred with styrene, phenylglycol would be produced:





The formation of an oily liquid, sparingly soluble in water, indicates the presence of organo-mercury compounds, while the appearance of a precipitate of mercurous acetate after prolonged steam-distillation indicates the formation of glycols. It is thought that both reactions occur, although in what proportion has not been determined, since either reaction brings about the desired result—viz., the changing of all olefinic compounds to compounds that are not steam-distillable.

II. The alkylbenzenes, after removal of the olefinic compounds, are nitrated by means of a suitable nitrating acid and the dinitro compounds are treated with acetone and potassium hydroxide in accordance with the Bost-Nicholson color test (2). In this reaction the color first formed by dinitromonoalkylbenzene is a deep blue, while the color formed by dinitro-*o*-xylene is a deep green. The green color in the presence of the blue cannot be satisfactorily measured by a colorimeter. However, advantage is taken of the fact that the blue color gradually changes to a purplish-red under the influence of alkali, while the green color remains and is measured by the photoelectric colorimeter. To hasten the change of the blue color and make the red less intense, the reaction is modified by the addition of monoethanolamine.

#### REAGENTS

**Propylene Glycol.** This reagent should be of a good grade, free from volatile impurities other than water.

**Mercuric Acetate Solution.** Dissolve 320 grams of reagent grade mercuric acetate (anhydrous) in 800 ml. of distilled water (cold, to keep formation of mercuric oxide at a minimum). Filter into a 1000-ml. glass-stoppered bottle. On long standing a small amount of mercuric oxide will form, which may be disregarded.

**Ethylbenzene, xylene-free.**

**Potassium Hydroxide, aqueous 50% solution.** Dissolve 75.0 grams of reagent grade potassium hydroxide pellets in 75.0 ml. of distilled water.

***o*-Xylene.** The xylene used for standards in this investigation was approximately 92% *o*-xylene, with 1 to 2% of *p*-xylene and approximately 6% of *m*-xylene.

**Nitrating Acid.** Mix equal parts, by volume, of concentrated c.p. nitric and sulfuric acids. Keep available sufficient of a single lot of this mixture to complete all determinations in a given series of analyses.

**Nitric Acid, concentrated c.p.**

**Acetone, reagent grade.** In this case also the accuracy of the colorimetric determination is enhanced if the reagent is kept in a single lot of consistent quality, and it should be used in connection with the same lot of nitrating acid.

**Diethyl Ether, U.S.P.**

**Monoethanolamine.** Use a technical grade, from a single lot of consistent quality and in connection with the same lot of nitrating acid.

#### APPARATUS

A 300-ml. Florence flask (flat-bottomed, long-necked, with vial mouth) is fitted with a glass standpipe and connected with a 250-ml. glass-stoppered Erlenmeyer flask. The Erlenmeyer is connected with an upright Liebig condenser fitted with an adapter having a tip of such size that it can be inserted into the neck of a Babcock milk testing bottle. The latter is the standard type with the neck graduated from 0 to 8%, each numbered graduation representing 0.20 ml. The diameter and length of the glass tubing connections should be kept small to aid in collecting the organic distillate without distilling a large volume of water. Rubber stoppers are used to complete the connections, and replaced from time to time, since they slowly deteriorate.

A shaker on which 250-ml. Erlenmeyer flasks may be placed is needed. A second shaker is required, to which is attached a small box with a snug hinged cover and painted black inside. A sponge-rubber mat with openings to accommodate two 30-ml. (1-ounce) bottles is placed in this box. The bottles are the narrow-mouthed

Table I. Sample Size

<i>o</i> -Xylene %	Sample Ml.	Alkylbenzene Ml.
Nil-0.10	5.0	0.15-0.20
0.10-0.5	5.0	0.20-0.25
0.50-1.0	3.0	0.2-0.3
1.0-3.0	3.0	0.4-0.6
3.0-6.0	3.0	0.6-0.8
6.0-20	2.0	0.5-0.7
20-50	1.0	0.4-0.9

French square type, fitted with silver foil-lined screw caps. In this investigation the second shaker was a Fisher Scientific Co. model No. 6-213A, oscillating 275 to 285 times per minute.

Another small box with black interior is required. Any small cardboard box with cover will do. In this investigation the box was a cube measuring 12.5 cm. (5 inches) on the side.

A photoelectric colorimeter equipped with the proper filters is required. A Lumetron model 402-EM instrument, fitted with a B660 filter, was used in this investigation. Of those available, this filter was found to be most efficient in preventing interference from the red color of dinitroethylbenzene, etc., at the same time allowing full measurement of the green color of dinitro-*o*-xylene.

Other necessary apparatus includes capillary-tipped pipets made from medicine droppers, an interval timer or stopwatch, and a centrifuge for the Babcock bottles.

#### SAMPLE SIZE

If the mixture is predominantly alkylbenzene, a 1-ml. sample is satisfactory.

With samples consisting largely of unsaturates, it is desirable to strike a balance between sample size and the volume of alkylbenzenes distilled over, depending on the percentage of *o*-xylene. When the *o*-xylene content is low, the smallest practicable amount of ethylbenzene is added to the sample to obtain a maximum concentration of *o*-xylene for the colorimetric determination. When the *o*-xylene content is high, more ethylbenzene should be added to increase the volume of alkylbenzenes, thus preventing too high a concentration of *o*-xylene. If sufficient or more than sufficient alkylbenzene is already present, no ethylbenzene is added. Table I serves as a guide for the proper sample size.

#### PROCEDURE

When a sample contains more than 99% styrene and less than 0.5% *o*-xylene, the procedure is as follows: Place 60 ml. of propylene glycol in a clean 250-ml. glass-stoppered Erlenmeyer flask and add, by means of a pipet, 5.0 ml. of the styrene sample. Determine the sample weight by weighing a similar amount. Add 0.20 ml. of xylene-free ethylbenzene to act as carrier for the *o*-xylene, then add 75 ml. of the mercuric acetate solution. Stopper the flask, securing the stopper firmly with friction tape, and shake vigorously on a shaking machine for 2 hours.

Connect the flask with the distillation apparatus and steam-distill the ethylbenzene and *o*-xylene into the Babcock bottle. Boil the water in the steam generator before heating the contents of the Erlenmeyer flask, in order to shorten the time the solution is hot while the alkylbenzenes are distilled. Continue the distillation until the bottle is about one-third full, or until just before the appearance of crystals of mercurous acetate, which rarely occurs before 35 to 40 ml. of distillate have been collected. Fill the Babcock bottle with distilled water and centrifuge at 1500 r.p.m. for 5 minutes. Measure and record the volume of the alkylbenzene layer. By means of a capillary-tipped dropping pipet transfer the alkylbenzene layer to a small vial, to be used as needed. The balance of the analysis should be completed within one working day, since the dinitro compounds decompose upon long standing.

Using a 0.1-ml. serological pipet graduated in 0.01 ml., transfer 0.050 ml. of the alkylbenzene mixture in the vial into the neck of a clean, dry 200-ml. volumetric flask, and wash immediately into the flask with 10.0 ml. of nitrating acid mixture. Shake the flask for a few seconds, then allow it to stand for 1 hour with occasional shaking, and finally allow it to cool in an ice bath.

Gradually add 25 to 30 ml. of distilled water, while shaking the flask, then add 10.0 ml. of concentrated nitric acid to dissolve the nitro compounds or to keep them in homogeneous suspension. Fill the flask to the mark with distilled water. Accurately pipet a 5.0-ml. aliquot of this solution into a small separatory funnel containing 10 ml. of distilled water. Make the solution in the funnel alkaline with 1.0 ml. of 50% potassium hydroxide; if the solution is made too basic the subsequent development of the colors may be lessened. Extract the alkaline solution with 10



Table II. Analyses of Known Solutions

<i>o</i> -Xylene Added %	<i>o</i> -Xylene from Stock Solution %	Total <i>o</i> -Xylene Present %	<i>o</i> -Xylene Found %
0.04	0.08	0.12	0.09, 0.13, 0.12
0.37	0.08	0.45	0.41, 0.42
3.69	0.08	3.77	3.82, 3.57, 3.73
10.8	0.07	10.9	11.0, 10.9
48.8	0.04	48.8	49.0, 48.2

% *o*-xylene in styrene for stock solution = 0.06, 0.07, 0.08, 0.08.  
% *o*-xylene in stock solution = 0.08, 0.09.

ml. of diethyl ether, and again with 5 ml. of ether, collecting the extracts in a clean, dry 1-ounce narrow-mouthed French square bottle. Gently evaporate the ether on a steam bath, leaving the nitro compounds and the water which was dissolved in ether. Take care to evaporate just the ether; evaporation of all or part of the water will lessen the intensity of the colors.

Pipet 20.0 ml. of acetone and 1.0 ml. of monoethanolamine into the 1-ounce bottle and add 2.0 ml. of 50% potassium hydroxide from a buret. Close the bottle tightly with the silver foil-lined screw cap, place it in the box on the shaker, and, starting the timer from zero, shake the bottle for 15.0 minutes. Leaving the timer in operation, transfer the bottle to the black cardboard box, and allow it to stand so that the caustic solution settles out of the colored acetone solution. After 17.0 minutes from the start of the shaking, fill a 10-mm., 10-ml. absorption cell with the solution and immediately read the per cent transmittance on the colorimeter, using a B660 and neutral gray filter with the transmittance of water at 100%. Record the first reading. Read the per cent *o*-xylene from a curve prepared by running the same procedure on known solutions of ethylbenzene and *o*-xylene.

#### CALCULATION

Calculate the per cent *o*-xylene in the original sample as follows:

$$\frac{A(B + C)DE}{\text{sample weight}} = \text{per cent (by weight) of } o\text{-xylene}$$

where *A* = volume per cent of *o*-xylene in alkylbenzenes. This figure is obtained by the colorimetric procedure.

*B* = ml. of alkylbenzenes. This figure is obtained by reading the volume of the alkylbenzenes distilled into the Babcock bottle.

*C* = volume increment. If only the volume of alkylbenzenes actually measured in the Babcock bottle (*B*, above) is used in calculating the per cent of *o*-xylene, the results will be low, owing to mechanical loss and the solubility of the alkylbenzenes in water. The volume of this loss may be obtained by running several known synthetic samples containing up to 5 to 6% of *o*-xylene, using the same *o*-xylene that was used in making the curve, and adding empirically the volume increment necessary to obtain the correct answer. This volume increment is constant, and with the apparatus and Babcock bottles used in this procedure was found to be 0.05 ml.

*D* = correction factor. This factor is necessary when impure *o*-xylene is used in preparing the curve. In this investigation 92% *o*-xylene containing 6% *m*-xylene and 2% *p*-xylene was used, and the error introduced by the meta and para isomers in making up the curve was negligible, the correction factor being  $\frac{100}{92} = 1.09$ .

*E* = specific gravity of *o*-xylene. The figure 0.87 was used.

#### ANALYTICAL DATA

A stock solution was prepared by mixing together 175 ml. of styrene, 15 ml. of 1,4-vinylcyclohexene, and 5 ml. of ethylbenzene. Various solutions of known *o*-xylene content were prepared from this stock solution and *o*-xylene. Results of analyses of these known solutions are shown in Table II.

#### ERRORS INTRODUCED BY OTHER COMPOUNDS

Using pure chemicals, the colorimeter readings given by various compounds possibly occurring in the samples, as well as the calculated percentage error if 5.0% of the compound were present, are given in Table III. Since propylbenzene, isopropylbenzene, and diethylbenzene boil well above styrene and *o*-xylene, while benzene and toluene boil well below, styrene and *o*-xylene can be readily fractionated out.

#### DISCUSSION

In general, 15 ml. of mercuric acetate reagent are used for each milliliter of sample. This gives, roughly 1.5 moles of mercuric acetate per mole of sample, a sufficient excess. One exception is when the sample contains considerable 1,4-vinylcyclohexene; because this compound has two olefinic linkages, a greater proportion of mercuric acetate must be added. If the sample contains but a small amount of olefinic compounds, less reagent may be added if desired.

Usually the volume of propylene glycol added is the same as that of the mercuric acetate reagent. However, if a 5.0-ml. sample is required, 60 ml. of propylene glycol are added even though 75 ml. of mercuric acetate reagent are used. This cuts down the volume of liquid in the Erlenmeyer flask. Ethylene glycol may be used, but its solvent action is not so great.

The contents of the flask after shaking will be a clear, water-white solution on polymer-free samples. However, any sample that can be pipetted, even with great difficulty, does not contain enough polymer to affect greatly the accuracy of the determination if the *o*-xylene content is below 0.5%.

The precaution of boiling the water in the steam generator before heating the contents of the Erlenmeyer flask makes it possible to complete the steam-distillation before the occurrence of a secondary reaction in which mercurous acetate precipitates and a small amount of an oily liquid distills over, lessening the accuracy of the determination. A small amount of acetic acid distills over also, but it offers no interference.

In the colorimetric determination the first reading is taken because the colors produced slowly fade when subjected to light.

The smaller the percentage of *o*-xylene, the smaller is the absolute error introduced from reading the volume of alkylbenzenes and the greater is the sensitivity of the colorimetric curve; thus samples containing less than 0.2% of *o*-xylene and not more than 5% of alkylbenzenes can be determined with an accuracy of  $\pm 0.01\%$ . When greater accuracy is desired or lower concentrations are to be determined, samples may be fractionated and the analyses made on selected cuts. Less than 0.01% *o*-xylene can be detected in a given sample. When large amounts of alkylbenzenes are present it is advisable to fractionate the material and determine the *o*-xylene on the proper fractions.

Table III. Errors Introduced

Compound	Reading for Pure Compound	% Error if 5.0% Present
Ethylbenzene	93.6	Nil
Isopropylbenzene	95.4	-0.04
Propylbenzene	94.4	-0.02
Toluene	82.5	+0.28
Benzene	89.7	+0.09
Diethylbenzene (mixture of isomers?)	97.3	-0.09
<i>m</i> -Xylene <sup>a</sup>	100.0	-0.16
<i>p</i> -Xylene	97.7	-0.10

<sup>a</sup> *m*-Xylene containing 10% *o*-xylene gave a reading of 79.8 which, when corrected by use of the curve, indicates a reading of 100 for pure *m*-xylene.

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# Laboratory-Size Glass Circulating Evaporator

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THE evaporation of solutions for the purpose of reducing volume and concentrating solids is one of the more frequently encountered operations common to many academic and industrial laboratory research problems. Strangely enough, this unit operation has received very little critical attention and in most laboratories is carried out much as has been the custom of the last 30 years with a steam cone and round-bottomed flask as the basic apparatus. This paper describes a convenient and compact apparatus modeled along the lines of large industrial units commonly used for the evaporation of aqueous solutions to make possible recovery of dissolved solids.

The glass parts of the heat exchanger were constructed by sealing five 0.375-inch outside diameter (standard-wall 10-mm. Pyrex) pieces of tubing between two small bulbs. One tube was centered from bottom to bottom between the two bulbs and then four additional tubes were sealed in on the corners of an imaginary square with a diagonal equal to the diameter of the bulbs. The packing glands were the conventional gland, string packing, and follower type. The gland bodies were brazed to the end plates of the metal heat-exchanger jacket. Since only one end plate need be removed in order to assemble the heat exchanger, one end only was fixed to the metal jacket by means of six bolts on a 9-cm. (3.625-inch) bolt circle, this end of the heat exchanger jacket and the jacket end plate being fitted with companion flanges. The other end plate was brazed to the jacket.

Instead of the rubber sleeve connections shown in Figure 1, standard-taper ball and socket glass joints may be substituted. This aids somewhat in assembly and disassembly and removes danger of contamination from anything save glass.

The glass tubes shown entering the evaporator bowl and the centrifugal separator were bent at right angles to the line of entry in a plane parallel to the floor, in order that they might deliver vapor tangential to the wall and thus impart a rapid swirling motion to the vapor in these two parts of the apparatus.

In principle this evaporator functions as do any of the long-tube natural circulating evaporators found in the chemical industry. The liquor to be concentrated is fed into the evaporator below the level of the heat exchanger. The liquor in the heat exchanger tubes boils, and the vapor rising as froth forces slugs of liquor ahead of it at high velocity up the tubes and out into the disengaging space in the evaporator bowl. As the system repeats this process, the liquor is rapidly circulated from heat exchanger to bowl to return line to heat exchanger, etc. The distinctive advantage of this type of evaporator lies in the high coefficient of heat transfer realized, due to the great velocity of the liquid over the heating surface. The character of the flow of liquid and vapor through the tubes guarantees a thorough and rapidly repeated wetting of the whole heating surface.

To operate the apparatus, vacuum is applied to the receiver and liquid drawn up until the larger bowl is filled to within 2.5 or 5 cm. (1 or 2 inches) of the vapor inlet. The liquor feed line is then shut by means of a screw clamp on a section of rubber hose. The pressure is allowed to come to a minimum, or to some predetermined pressure if a vacuum controller is to be used. An intermittent leaks type of vacuum controller with an electrically actuated valve working from one of the conventional mercury-filled U-tube manostats is to be recommended, since the evaporator will operate much more smoothly at constant pressure. Steam is then turned on the jacket. The steam condensate line is conveniently placed below the surface of the water emerging from the surface condenser. No positive steam pressure is needed for aqueous solutions. The solution in the small tubes of the heat exchanger rapidly comes to boil and emerges from the top of the exchanger as a mixture of vapor and entrained liquor. The charge in the evaporator rapidly comes to its boiling point as the liquor circulates. The vapor and liquor emerging from the vapor outlet are given a circular motion by the bend in the end of the vapor outlet. The centrifugal force developed throws all drops of liquor or foam against the evaporator sides where they run down at once to the charge in the bowl. Any foam or entrainment not removed by the first centrifugal separator is taken out by the second.

## ADVANTAGES OF APPARATUS

The unit will closely duplicate results obtained with tube evaporators of commercial size. Protein or other food product solutions, almost impossible to handle in a flask because of excessive foaming, may be evaporated with little or no difficulty.

Bumping is eliminated in most cases. When it does occur it results rather in a surging action in the heat exchanger tubes. This can be overcome if desired by allowing a small stream of air bubbles to enter the system by cracking the clamp on the liquor inlet line, which in this case must now open to the atmosphere.

Evaporation is rapid as compared with the usual setup and extremely so for glass surfaces exposed to steam.

Heat-sensitive materials receive a minimum of damage.

The recovery of solids from a solution is satisfactory. Aqueous solutions of organic or inorganic material on being evaporated to where solids separate in general give small crystal size with but little tendency to crust on the inner surfaces of the evaporator. Crusting is apparently prevented by the scouring action of the rapidly circulating liquor.

The unit may be used for continuous evaporation. In this event the liquor inlet is used for continuous feeding of fresh liquor and the finished liquor is drawn off continuously through a T in the return down-leg line.

The unit may be easily dumped, cleaned, and refilled. Dump-

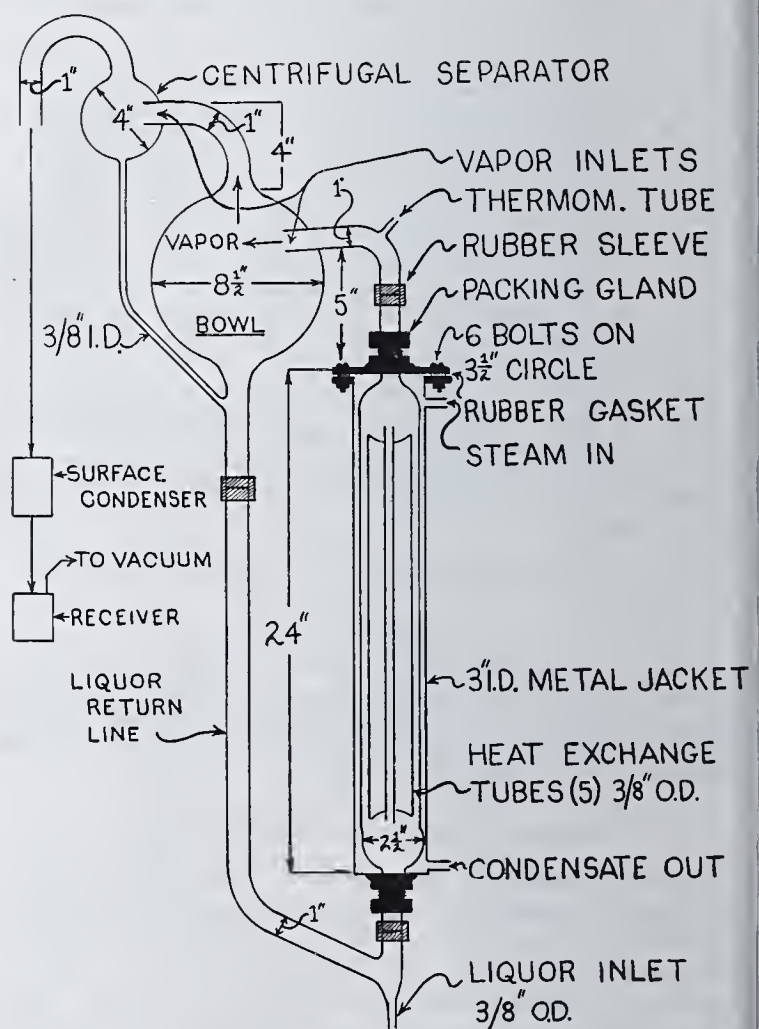


Figure 1. Diagram of Evaporator



Table I. Operation Characteristics

(Wall thickness of heat exchanger tubes,  $\frac{5}{16}$  inch)

Hg Pressure	Liquor Temp.	Steam Temp.	T	H <sub>2</sub> O Evapo- rated	Glass Surface	H <sub>2</sub> O Evapo- rated	B.t.u./Sq. Ft./Hour/ ° F.
Mm.	° F.	° F.		Lb./hr.	Sq. ft.	Lb./hr./ sq. ft.	
103	126	212	86	11.40	0.955	11.95	142
85	119	212	93	12.15	0.955	12.73	140
65	109.5	212	102.5	13.15	0.955	13.78	138.5
Operation of 12-liter flask on a steam cone							
97	123.8	212	88.2	10.35	1.88	5.5	66.2

ng merely requires the vacuum to be released, after which the charge will flow out under gravity. To recover the liquor or crystals adhering to the interior walls, it is only necessary to draw up about 50 to 100 cc. of water into the evaporator, allow the vacuum to build to a few hundred millimeters' pressure, and then suddenly open the inlet line. The rapid surge of air up through

the heat exchanger tubes throws the small amount of wash liquor around violently, and on releasing the vacuum, it drains out and carries with it the portion of the original charge remaining on the walls of the apparatus.

With pure water this evaporator has the operation characteristics shown in Table I.

The authors have several such units in use in their laboratories, ranging from several times the size shown to small units suitable for handling as little as 100 cc. For the smaller units the heat exchanger is usually made up with only one glass tube through the steam jacket. The jacket is commonly made from scrap thin-walled boiler tubes, with rubber stoppers replacing the two packing glands on the jacket.

This type of evaporator may be fabricated from standard Pyrex tubes and flasks, or may be purchased complete from suppliers of special glass apparatus. (Ace Glass, Inc., has indicated a willingness to fabricate such an apparatus in any size desired.)

## Identification of Some Important Unsulfonated Azo-2-naphthol Dyes

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A rapid and simple method has been developed for the identification of unsulfonated azo-2-naphthol dyes, by catalytic reduction of the azo bond, and the separation of the scission products with immiscible solvents, under controlled acid and alkaline conditions. Direct formation of the stable benzoyl derivatives of the reduction products precludes the necessity of isolating sensitive diamines and triamines.

**A**ZO dyes prepared from diazotized primary aromatic amines and 2-naphthol constitute an important series of commercial colors, and their identification is important to the dyestuff chemist. Several analytical methods have been proposed, among which are hydrogenation of the azo bond (2, 3, 10, 11, 12), scission of the azo link with fuming nitric acid (7, 8, 9), and schematic identification by means of immiscible solvents (4, 5, 6).

Whitmore and Revukas (10, 11) have shown the applicability of catalytic reduction to azo colors by the use of Raney nickel. Their procedure, however, involves moderately expensive equipment and consumes considerable time when nitrated dyes are hydrogenated, and they report that the isolation and identification of the reduction products are "laborious".

This paper proposes a rapid and simple method for the separation and characterization of the hydrogenation compounds which is based upon their differential solubility in water and ether under varying acid and alkaline conditions.

Cheronis and Koeck (1) have devised a simple semimicro hydrogenation apparatus, obtainable from the Wilkens-Anderson Company of Chicago, which the authors find very adaptable to the reduction of azo colors. Utilizing this outfit, it is possible to hydrogenate this series of dyes in peroxide-free dioxane (11), smoothly and quickly. Subsequent isolation of the reduction products, by the immiscible solvent procedure, and direct conversion into their benzoyl derivatives, eliminate the necessity of recovering the sensitive free amines.

Partial dehalogenation of chlorinated dyes, an inevitable accompaniment of all attempted reductions in neutral solvents,

was overcome by acidification of the peroxide-free dioxane before hydrogenolysis of the color.

### GENERAL PROCEDURE

**PURIFICATION OF SAMPLES.** The coloring matter is purified by crystallization from dioxane, and if or when necessary, water is added to this solvent to induce precipitation.

**PREPARATION OF REDUCTION PRODUCTS.** Freshly ground Adams-Voorhees platinum oxide (0.05 gram) is placed in the Cheronis hydrogenating unit, and the catalyst is suspended in 25 ml. of peroxide-free dioxane. Hydrogen gas, preferably from a tank, is bubbled through the suspension for 2 to 5 minutes, in order to convert the platinum oxide to colloidal platinum black.

Then 1 gram of dye and 2.5 ml. of concentrated hydrochloric acid are added to the platinum black suspension, the hydrogenating unit is heated by immersion in hot water, 80° to 90° C., and hydrogen gas is passed through the mixture at such a rate that continuous agitation is maintained. (Occasionally, the reduction products will clog the disperser, and it is convenient to have a clean one available for quick replacement. If a second unit is not on hand, it is necessary to remove the clogged disperser, and to clean it by immersion in a test tube containing hot dioxane. At or near the completion of the hydrogenolysis, it is advisable to wash down any dye particles adhering to the sides of the tube, with 5 to 10 ml. of dioxane.)

**SEPARATION OF THE REDUCTION PRODUCTS.** If a precipitate of the reduction products forms in the acidified dioxane solution, it is redissolved by the addition of 5 to 10 ml. of water and filtered into a 500-ml. Squibb separatory funnel. The filtrate is buffered with 250 ml. of a 5% sodium acetate solution, and the ether-soluble amino-2-naphthol, whose presence is indicated by a blue fluorescence, and any primary monoamine, are extracted with two successive 100-ml. portions of ether.

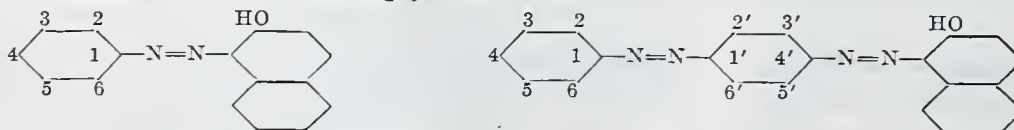
The aqueous layer, containing any water-soluble primary polyamine, is separated from the ether and intimately mixed with 5 ml. of benzoyl chloride. The combined ether fractions are washed with two 50-ml. volumes of water, to remove residual polyamine, and the washings are run into the benzoylation mixture. Then 10 grams of solid sodium hydroxide are added, sufficient to make the aqueous solution alkaline, and after standing at room temperature for 30 minutes, with frequent stirring, the mixture is placed on the steam bath to expel dissolved ether. The benzoyl derivative of the diamine or triamine, which separates as a solid, is filtered off, washed thoroughly with water, and crystallized from a suitable solvent.



Table I. Identification of Unsulfonated Azo-2-Naphthol Dyes

Dye	Melting Point		Reduction Products of Diazo Component	Melting Point of Benzoyl Derivative of Reduction Product of Diazo Component		Reduction Time Min.
	Observed (uncorrected) ° C.	Literature ° C.		Observed (uncorrected) ° C.	Literature ° C.	
1. Benzeneazo-2-naphthol <sup>a, b</sup>	131-2	131	Aniline	162-3 <sup>c</sup>	163	15
2. 2-Methylbenzeneazo-2-naphthol	132-3	128	<i>o</i> -Toluidine	144-5 <sup>c</sup>	145-6	20
3. 4-Ethoxybenzeneazo-2-naphthol	133-4	140	<i>p</i> -Phenetidine	172-3 <sup>c</sup>	173	25
4. 4-Methylbenzeneazo-2-naphthol	133-4	130	<i>p</i> -Toluidine	157-8 <sup>c</sup>	157	10
5. 4-Methoxybenzeneazo-2-naphthol	140-1	139	<i>p</i> -Anisidine	155-6 <sup>c</sup>	156	30
6. 3-Methylbenzeneazo-2-naphthol	140-1	140	<i>m</i> -Toluidine	123-4 <sup>c</sup>	125	15
7. 2-Ethoxybenzeneazo-2-naphthol	142-3	145	<i>o</i> -Phenetidine	Oil	...	20
8. 2,5-Dimethylbenzeneazo-2-naphthol	152-3	156	<i>p</i> -Xylidine	147-8 <sup>c</sup>	140	15
9. 2,4,5-Trimethylbenzeneazo-2-naphthol	155-7	...	Pseudocumidine	169-70 <sup>c</sup>	162	15
10. 2,4-Dimethylbenzeneazo-2-naphthol <sup>d</sup>	160-1	166	<i>m</i> -Xylidine	193-4 <sup>c</sup>	192	15
11. 3-Chlorobenzeneazo-2-naphthol	160-1	...	<i>m</i> -Chloroaniline	120-21 <sup>c</sup>	118-20	30
12. 4-Chlorobenzeneazo-2-naphthol <sup>e</sup>	161-2	160	<i>p</i> -Chloroaniline	191-2 <sup>c</sup>	192-3	30
13. 2-Chlorobenzeneazo-2-naphthol	167-8	167	<i>o</i> -Chloroaniline	104-5 <sup>c</sup>	99-101	30
14. 2-Methyl-4-chlorobenzeneazo-2-naphthol	170-1	172	2-Methyl-4-chloroaniline	142-3 <sup>c, f</sup>	...	90
15. 2-Methyl-5-chlorobenzeneazo-2-naphthol	176-7	...	2-Methyl-5-chloroaniline	171-2 <sup>c, f</sup>	...	30
16. 3-Methylbenzeneazo-3'-methylbenzeneazo-2-naphthol	175-7	...	<i>m</i> -Toluidine	123-4 <sup>c</sup>	125	20
17. 2-Methoxybenzeneazo-2-naphthol	180-1	180	2-Methyl- <i>p</i> -phenylenediamine	301-2 <sup>g, h</sup>	...	10
18. 2-Naphthaleneazo-2-naphthol	181-2	174	<i>o</i> -Anisidine	Oil	59.8	20
19. 2,5-Dichlorobenzeneazo-2-naphthol <sup>i</sup>	183-4	...	2-Naphthylamine	160-1 <sup>i</sup>	161	20
20. 2-Methylbenzeneazo-2'-methylbenzeneazo-2-naphthol	188-9	...	2,5-Dichloroaniline	119-20 <sup>c</sup>	120	30
21. 3-Nitrobenzeneazo-2-naphthol	196-7	194	<i>o</i> -Toluidine	144-5 <sup>c</sup>	145-6	30
22. Benzeneazobenzeneazo-2-naphthol	199-200	202	2-Methyl- <i>p</i> -phenylenediamine	301-2 <sup>g, h</sup>	...	40
23. 2-Nitro-4-methoxybenzeneazo-2-naphthol	206-7	...	<i>m</i> -Phenylenediamine	241-2 <sup>g</sup>	240	90
24. 5-Nitro-2-methylbenzeneazo-2-naphthol	210-11	206	Aniline	162-3 <sup>c</sup>	163	...
25. 2-Nitrobenzeneazo-2-naphthol	213-4	212	<i>p</i> -Phenylenediamine	338-9 <sup>g</sup>	Over 300	65
26. 1-Naphthaleneazo-2-naphthol	232-3	224	4-Methoxy- <i>o</i> -phenylenediamine	251-2 <sup>g</sup>	251-2	45
27. 4-Nitro-2-methylbenzeneazo-2-naphthol	251-2	248	1-Methyl-2,4-phenylenediamine	225-6 <sup>g</sup>	224	30
28. 4-Chloro-2-nitrobenzeneazo-2-naphthol <sup>k</sup>	255-6	252	<i>o</i> -Phenylenediamine	303-4 <sup>g</sup>	301	20
29. 2,5-Dimethylbenzeneazo-2',5'-dimethylbenzeneazo-2-naphthol	263-4	...	1-Naphthylamine	158-9 <sup>g</sup>	158, 161-2	30
30. 2-Nitro-4-methylbenzeneazo-2-naphthol	273-4	278	2-Methyl-1,4-phenylenediamine	301-2 <sup>g, h</sup>	...	55
31. 2-Chloro-4-nitrobenzeneazo-2-naphthol <sup>m</sup>	289-90	282	4-Chloro- <i>o</i> -phenylenediamine	226-7 <sup>d</sup>	230	120
32. 2,4-Dinitrobenzeneazo-2-naphthol <sup>n</sup>	312-13	302	<i>p</i> -Xylidine	147-8 <sup>c</sup>	148	...
			2,5-Dimethyl- <i>p</i> -phenylenediamine	311-12 <sup>g, l</sup>	...	150
			4-Methyl- <i>o</i> -phenylenediamine	263-4 <sup>g</sup>	263-4	50
			2-Chloro- <i>p</i> -phenylenediamine	239-40 <sup>c</sup>	228	50
			1,2,4-Triaminobenzene	278-9 <sup>g, p</sup>	260	...

<sup>a</sup> The nomenclature follows the numbering systems shown below:



<sup>b</sup> The benzoyl derivative of amino-2-naphthol, from all compounds investigated, was recrystallized from ethanol, M.P. observed 232-3° C. (uncorrected) M.P. literature, 226.5°, 235.5° C.

<sup>c</sup> Solvent, ethanol-water.

<sup>d</sup> To differentiate further between compounds 10 and 12, analyze for presence of halogen by any accepted procedure.

<sup>e</sup> Monobenzoyl derivative, C<sub>8</sub>H<sub>7</sub>CH<sub>2</sub>Cl.NHCOC<sub>6</sub>H<sub>5</sub>. Chlorine calculated, 14.44%; found, 14.40%.

<sup>f</sup> Monobenzoyl derivative, C<sub>8</sub>H<sub>7</sub>CH<sub>2</sub>Cl.NHCOC<sub>6</sub>H<sub>5</sub>. Chlorine calculated, 14.44%; found 14.47%.

<sup>g</sup> Solvent, acetic acid.

<sup>h</sup> Dibenzoyl derivative, C<sub>8</sub>H<sub>7</sub>CH<sub>2</sub>(NHCOC<sub>6</sub>H<sub>5</sub>)<sub>2</sub>. Nitrogen calculated 8.48%; found, 8.50%.

<sup>i</sup> Solvent, ethanol.

<sup>j</sup> 2,5-Dichloroaniline did not wash out of ether layer with *N* hydrochloric acid. It was isolated by evaporating ether and crystallizing residue from ethanol; M.P. 49° C. (uncorrected).

<sup>k</sup> Nearly all the diazo component was found as 4-chloro-2-nitraniline in ether layer, M.P. 117-18° C. (uncorrected). Its benzoyl derivative melted at 131-2° C. (uncorrected).

<sup>l</sup> Dibenzoyl derivative, C<sub>8</sub>H<sub>7</sub>(CH<sub>3</sub>)<sub>2</sub>(NHCOC<sub>6</sub>H<sub>5</sub>)<sub>2</sub>. Nitrogen calculated, 8.14%; found, 8.18%.

<sup>m</sup> About one half of diazo component was found in ether layer as 2-chloro-4-nitraniline, M.P. 107-8° C. (uncorrected). Its benzoyl derivative melted at 157-8° C. (uncorrected).

<sup>n</sup> About one half of diazo component was found unreacted to triamine form and was isolated from ether layer as 2,4-dinitraniline, M.P. 178-9° C. (uncorrected).

<sup>o</sup> Solvent, acetic acid-water.

<sup>p</sup> Benzoyl derivative of triamine (1,2,4-triaminobenzene) isolated from reduction of *p*-ethoxybenzeneazo-*m*-phenylenediamine, had same M.P.

Amphoteric amino-2-naphthol is separated from the primary monoamine, by removing the former with four successive 50-ml. portions of 2% sodium hydroxide solution, followed by two 50-ml. volumes of water, and is isolated as the benzoyl derivative by running the alkaline extracts into 5 ml. of benzoyl chloride, stirring vigorously after each addition. Dissolved ether is eliminated by heating on the steam bath, and after cooling to room temperature, the benzoyl derivative is filtered off, washed thoroughly with water, and crystallized from ethanol.

The ether solution, containing the primary monoamine, is shaken with four 50-ml. portions of *N* hydrochloric acid, followed by a 50-ml. volume of water. Dissolved ether is expelled by heating, and the acid solution is cooled. Ten grams of solid sodium hydroxide are added to liberate the free amine, and the mixture is treated with 5 ml. of benzoyl chloride. Upon vigorous agitation, the benzoyl derivative separates as a pasty mass, which is allowed to stand overnight. It is then filtered off, washed thoroughly with water, and crystallized from a suitable solvent.

If the melting point of the purified dye indicates the presence of 2,5-dichlorobenzeneazo-2-naphthol, 2,4-dinitrobenzeneazo-2-naphthol, 2-chloro-4-nitrobenzeneazo-2-naphthol, or 4-chloro-2-nitrobenzeneazo-2-naphthol, the residual ether is evaporated on

a steam bath, and the residue is identified according to the directions given in the footnotes to Table I.

#### ACKNOWLEDGMENT

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# Estimation of Vitamin C in Presence of Iron Salts

## Stepwise Determination of Vitamin C and Ferrous Iron with Dichlorophenolindophenol

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REPORTS on the reductive interference of ferrous iron with the dichlorophenolindophenol reagent used in the estimation of vitamin C have appeared in the literature and procedures have been devised to minimize and remove this interference (2-5).

During the course of studies in this laboratory on isolated vitamin C-iron salt systems the authors have found that ferrous and ferric iron interferences can be avoided if a suitable medium is chosen for the dye titration. Thus vitamin C can be determined in the presence of ferrous iron if acetic acid is employed as the titration medium and in the presence of ferric iron if metaphosphoric acid is present in the medium. The observation that ferrous iron reduces dichlorophenolindophenol in the presence of metaphosphoric acid provides a basis for the stepwise determination of vitamin C and ferrous iron in the same aliquot.

Whether or not these observations may be applied in toto or in part to biological systems, pharmaceuticals, and food products where other interferences (3) in addition to iron may be present remains to be determined.

### REAGENTS

**Ferrous Iron Solution.** Two grams of dried ferrous sulfate powder were dissolved in 160 ml. of distilled water containing 5 ml. of concentrated sulfuric acid by warming gently on the steam bath. The solution was treated with a rapid current of hydrogen sulfide for 0.5 hour, followed by a stream of nitrogen, and then made up to a volume of 1000 ml. with distilled water. An aliquot titrated with 0.01 *N* potassium permanganate contained 0.64 mg. of ferrous iron per ml.

**8% Acetic Acid,** 80 ml. of glacial acetic acid made up to liter with distilled water.

**Table I. Influence of Medium on Reduction of Dichlorophenolindophenol by Ferrous Iron**

(0.64 mg. of ferrous iron present)

Titration Medium <sup>a</sup>	Dichlorophenolindophenol Required for End Point, Ml.
8% acetic acid	0.05
8% metaphosphoric acid	9.1
8% metaphosphoric acid	9.2
Citrate reagent (Bessey)	Slowly fading end point
8% acetic acid adjusted to pH 3.5 with NaOH	0.05
8% acetic acid with 0.025% metaphosphoric acid present	3.2
	Reduction very slow and not reproducible
8% acetic acid with 0.15% metaphosphoric acid present	5.0
8% acetic acid with 0.5% metaphosphoric acid present	7.3
8% acetic acid with 1.0% metaphosphoric acid present	9.0
8% acetic acid with 3% metaphosphoric acid present	9.1

<sup>a</sup> Initial volume 25.0 ml.

**Table II. Influence of Medium on Titration of Vitamin C with Dichlorophenolindophenol in Presence of Ferric Iron**

(1.00 mg. of vitamin C and 0.75 mg. of ferric iron<sup>a</sup> present in all cases)

Titration Medium <sup>b</sup>	Dichlorophenolindophenol Required for End Point, Ml.
8% acetic acid	5.9 <sup>c</sup>
8% acetic acid with 3% metaphosphoric acid present	9.2
3% metaphosphoric acid	9.3
6% metaphosphoric acid	9.2

<sup>a</sup> From ferric ammonium sulfate standardized by reduction and subsequent titration with KMnO<sub>4</sub>.

<sup>b</sup> Initial volume 25.0 ml.

<sup>c</sup> Not reproducible.

6% Metaphosphoric Acid, 60 grams of crushed metaphosphoric acid sticks dissolved in distilled water and made up to liter. When not in use this solution was kept in the refrigerator.

Citrate and Metaphosphoric Acid Buffer, as described by Bessey (1)

Dichlorophenolindophenol Dye Solution, 50 mg. of ether-extracted dichlorophenolindophenol dissolved in 200 ml. of distilled water to which 42 mg. of sodium bicarbonate had been added. When not in use the dye was stored in the refrigerator.

**Table III. Estimation of Vitamin C and Ferrous Iron**

Sample	Total Dye <sup>a</sup>		Found	
	20 ml. of 8% acetic acid	Subsequent addition of 10 ml. of 6% metaphosphoric acid	Vitamin C	Ferrous iron
	Ml.	Ml.	Mg.	Mg.
0.64 mg. of ferrous iron	0.05	9.1	0.0	0.63
1.0 mg. of vitamin C	9.2	9.2	0.99	0.00
1.0 mg. of vitamin C and 0.64 mg. of ferrous iron	9.3	18.6	1.01	0.64
Vitamin C <sup>b</sup> capsule containing FeSO <sub>4</sub>	12.8	21.6	34.7	15.1

<sup>a</sup> Dye standardized against U.S.P. vitamin C.

<sup>b</sup> Vitamin capsule containing 35 mg. of vitamin C and 15 mg. of iron, diluted to 25 ml., 1-ml. aliquot used for titration. Capsule found by oxidation and colorimetric estimation with thiocyanate to contain 15.0 mg. of iron

### EXPERIMENTAL AND DISCUSSION

Table I shows that ferrous iron reduces dichlorophenolindophenol in the presence of metaphosphoric acid. This also happens when phosphoric acid is present and can be explained on the basis of the increase in reduction potential of the ferrous-ferric system when the effective ferric-ion concentration is reduced by complex formation with metaphosphate or phosphate ions.

The reverse of the above phenomena takes place in the presence of ferric iron. Here, as can be seen from Table II, the reduced dye is reoxidized in acetic acid medium but not in the presence of metaphosphoric acid. This reoxidation takes place at a much slower rate than the corresponding reduction by ferrous iron and at the present time is being investigated as the basis for a colorimetric estimation of iron.

From the above experiments it was apparent that vitamin C and ferrous iron could be determined stepwise on one aliquot by first titrating in acetic acid, then adding metaphosphoric acid and continuing the titration. Control analyses and the analysis of a vitamin capsule containing vitamin C and ferrous sulfate are given in Table III.

### SUMMARY

Ferrous sulfate reduces dichlorophenolindophenol in the presence of metaphosphoric acid. Ferric iron oxidizes reduced dichlorophenolindophenol in acetic acid medium. Vitamin C and ferrous iron can be determined on one sample by stepwise titration with dichlorophenolindophenol.

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# Determination of Small Amounts of Zinc

## By Measurement of Fluorescent Turbidities

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A rapid and accurate method for the determination of zinc by measuring the fluorescence of a turbidity of zinc 8-hydroxyquinolate is described. The range of the method is from 0.05 to 0.60 mg. and the accuracy is about 0.02 mg. The influence of variations in temperature, filters, method of procedure, amount of reactants added, and extraneous salts has been investigated. Other ions precipitated by 8-hydroxyquinoline in acetic acid-acetate solutions interfere. Zinc can be determined in the presence of magnesium by the method if calibration curves are constructed using approximately the amount of magnesium present in the unknowns.

**Z**INC forms a precipitate with 8-hydroxyquinoline which fluoresces brilliantly. It has been found possible to produce stable, reproducible turbidities of zinc 8-hydroxyquinolate and to measure the turbidity accurately by means of its fluorescence. Most fluorescence methods developed previously have dealt with true solutions.

The method developed by White and Lowe (14) for aluminum using morin as the reagent is believed to be due to the formation of a colloidal solution of  $\text{Al}(\text{C}_{15}\text{H}_9\text{O}_7)_3$  (11).

A number of workers (4, 5) have used the fluorescence of the 8-hydroxyquinoline precipitates for qualitative detection of zinc and other metals. Lutz has described (7) a method for determination of zinc by measurement of the fluorescence of zinc salts with urobilin. Sandell (10) has published a method for the fluorometric determination of gallium as the 8-hydroxyquinoline complex dissolved in chloroform and suggests (9) that zinc could be determined similarly.

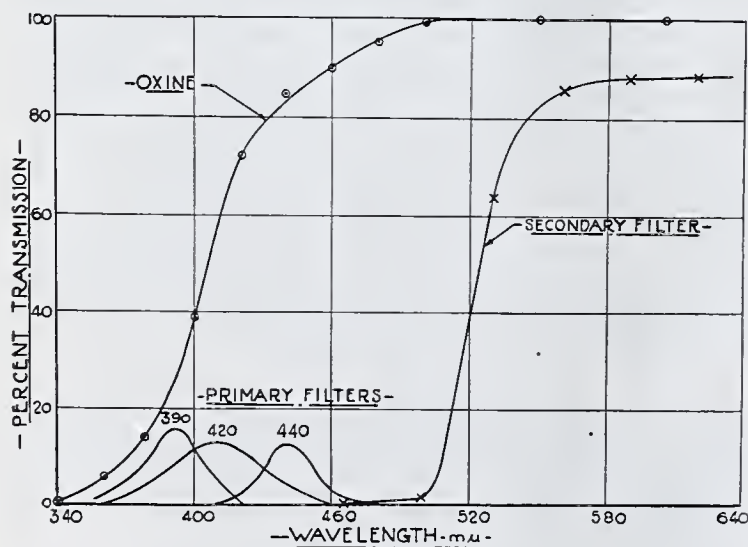


Figure 1. Transmission of Filters and Reagent

Zinc has been determined colorimetrically or nephelometrically by numerous methods. Among the most useful are the colorimetric methods employing dithizone (9); these are extremely sensitive and are employed for amounts of zinc ranging from a microgram up to about 1 mg. These methods require extraction with an organic solvent such as carbon tetrachloride. The nephelometric determination of zinc with ferrocyanide (2, 3) covers the range of zinc from about 0.1 to 5 mg. but the average error is about  $\pm 0.05$  mg. (1). Zinc can also be determined turbidimetrically (15) or nephelometrically (8) as the sulfide.

Teitelbaum (13) has described an indirect colorimetric method in which zinc is precipitated as the 8-hydroxyquinolate com-

plex salt, the precipitate is then dissolved in acid, and the liberated 8-hydroxyquinoline is determined by its reducing action on Folin's reagent. The method is applicable to about 0.008 to 0.09 mg. of zinc with an accuracy of 2 to 5%.

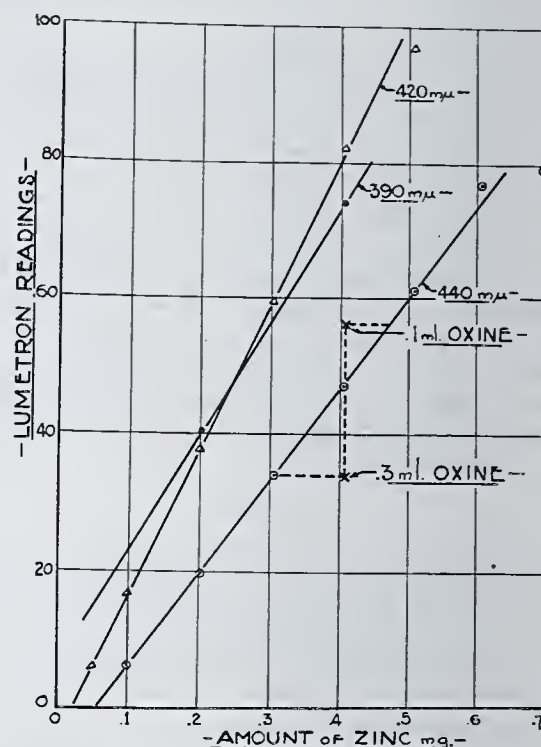


Figure 2. Influence of Filters and Amount of Reagent

The determination of zinc by measurement of the fluorescence of 8-hydroxyquinoline turbidities as described in this paper covers the range from about 0.02 to 0.60 mg. of zinc per 50 ml. of final solution and is accurate to about 0.02 mg. Thus larger quantities of zinc can be determined than is possible by most similar methods, without dilution or the necessity of taking aliquot portions. The procedure is simple and extremely rapid, since no filtrations nor extractions with immiscible solvents are required. The method is, however, subject to the many interferences encountered with all procedures involving 8-hydroxyquinoline.

In producing reproducible turbidities, a large number of factors must be considered (6), such as the temperature, concentration of other salts present, manner of mixing, time of standing, concentration and order of solutions mixed, presence or absence of protective colloids, etc. All these factors must be controlled and once a method has been established all details of procedure must be adhered to rigidly. The measurement of zinc 8-hydroxyquinolates by fluorescence of turbidities is further complicated by the fact that the reagent, which must be added in slight excess absorbs the light which excites the fluorescence. Proper filters must be employed in the fluorescence meter and the concentration of reagent must be controlled.

### REAGENTS

A standard solution of zinc chloride was prepared by dissolving 4.0982 grams of c.p. zinc metal (low in lead, iron, and arsenic) in 35 ml. of concentrated hydrochloric acid and diluting the solu-



tion to 2 liters with distilled water. A solution containing 0.02049 mg. of zinc per ml. was prepared by diluting 10 ml. of the above standard solution to 1 liter.

A 5% solution of 8-hydroxyquinoline was prepared by dissolving 5 grams of c.p. 8-hydroxyquinoline in 12 grams of glacial acetic acid and diluting to 100 ml. with distilled water.

A 2% solution of gum arabic was prepared by grinding 2 grams of gum arabic in a mortar until fine, and dissolving in water to make 100 ml. The solution was filtered if not clear.

A 2 N ammonium acetate solution was obtained by dissolving 154 grams of crystallized ammonium acetate in water to make 1 liter.

A standard dichlorofluorescein (or fluorescein) solution was prepared in the following manner: A 0.1% alcoholic solution of dichlorofluorescein (such as is usually employed as an adsorption indicator) was added drop by drop to 1 liter of water until the resulting solution had a fluorescence approximately the same as that of a turbidity produced from 0.30 mg. of zinc in the manner described below. About 0.35 ml. of dichlorofluorescein solution was required. A more concentrated solution may be employed, if desired. The standard solution is stable for weeks.

#### PROCEDURE

Amounts of standard zinc solution containing between 0.05 and 0.50 mg. of zinc were placed in a 50-ml. volumetric flask, and 5 ml. of 2 N ammonium acetate and 2 ml. of 2% gum arabic solution were added. The mixture was diluted with water to approximately 45 ml. and mixed by swirling the flask. Using a serological pipet, 0.20 ml. of 5% 8-hydroxyquinoline solution was added. The solution was diluted to the mark with distilled water and mixed by gently shaking the flask. The turbid solution was poured into the cell of the fluorescence meter and the per cent fluorescence was measured.

A Lumetron Model 402EF fluorescence meter was employed in this investigation. The 25-ml. cells supplied for this instrument were used for all measurements. The instrument was standardized by setting the fluorescence emitted by the standard dichlorofluorescein solution at 50.0 and that emitted by a blank containing all reagents but no zinc equal to 0.0. If a more concentrated solution of dichlorofluorescein or known zinc turbidity is employed as standard the fluorescence is adjusted to 100.0. A narrow band filter of 420  $m\mu$  maximum transmission obtained from the Photovolt Corporation was employed in the primary beam. Since the fluorescent light is greenish-yellow, the yellow secondary filters furnished with the instrument for riboflavin determinations were employed in front of the measuring cells. As can be seen in Figure 1, the secondary filters will effectively absorb any of the primary light which might be scattered or reflected toward the measuring cells.

Measurements were made on the turbidities 2 to 3 minutes after the reagent had been added. The exact time before measurement is not critical. The turbidities remain the same for at least 25 minutes. A calibration curve was constructed by plotting the concentration of zinc against the per cent of fluorescence as read on the instrument. Unknown concentrations are read from this calibration curve.

#### INFLUENCE OF PRIMARY FILTER AND CONCENTRATION OF REAGENT

The fluorescence of the zinc turbidity is more intense when excited by light of shorter wave lengths in the region 460 to 350  $m\mu$ . Above about 460  $m\mu$  the fluorescence disappears or is too small to be measurable. The reagent, however, has a strong absorption band in the near ultraviolet and absorption, as measured on a Beckman spectrophotometer, is appreciable even at 460  $m\mu$ . The per cent transmission of a 1-cm. layer of 0.002 M 8-hydroxyquinoline in acetic acid-ammonium acetate solution (pH = 5.9) is shown in Figure 1. The per cent transmission values for the filters investigated are also shown in Figure 1.

In Figure 2 are plotted the readings of the Lumetron against amount of zinc ion present, using three different primary filters. An intermediate amount of zinc was selected and the effect of adding 0.1 and 0.3 ml. of 8-hydroxyquinoline solution instead of the usual 0.2 ml. was determined. The method of calculation for filter 440  $m\mu$  is shown in Figure 2. At 440  $m\mu$  the error in milligrams of zinc per 0.1-ml. variation in amount of reagent added is 0.08 mg., at 420  $m\mu$  the value is 0.06 mg., and at 390  $m\mu$  the value is 0.13 mg. The most suitable filter is the one showing the greatest sensitivity to the amount of zinc present with the least variation due to a change in reagent concentration.

The 420  $m\mu$  filter is the most suitable and was employed in all further investigations.

#### INFLUENCE OF TEMPERATURE

Calibration curves were constructed using the standard zinc solution for 15°, 24°, and 35° C. The solutions were allowed to come to the specified temperature before the turbidities were produced. The solutions were diluted to volume with water of the same temperature. The comparisons were all made at the temperature of the instrument, approximately 25° C. The curves are shown in Figure 3. At a level of about 0.3 mg. of zinc a

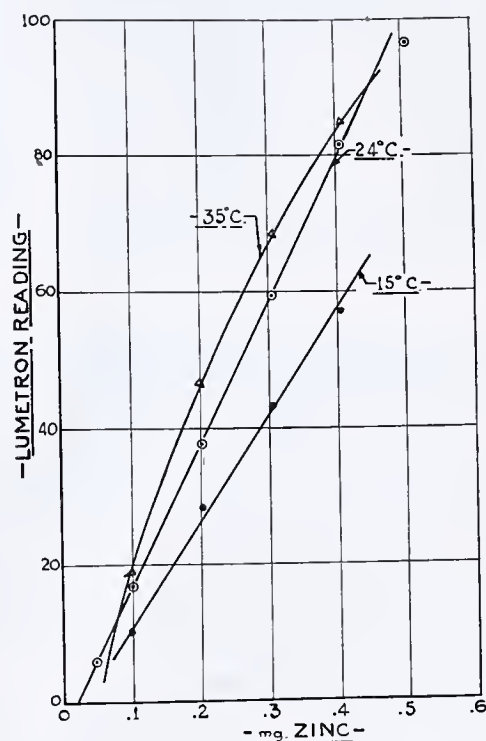


Figure 3. Effect of Temperature

change in temperature, especially a decrease in temperature, of about 2° C. will cause an error of 0.02 mg. of zinc or less. The temperature of the solutions should be kept within  $\pm 2^\circ$  C. of that used in the establishment of the calibration curves. Frequently room temperature will suffice.

#### INFLUENCE OF ADDED SALTS

Turbidities were prepared containing 0.205 mg. of zinc plus varying amounts of added salts per 50-ml. volume. As little as 0.2 mg. of ferric ion reduces the fluorescence to zero. No more than about 3 micrograms of ferric ion can be tolerated. Aluminum and other ions precipitated in acetic acid-acetate solutions by 8-hydroxyquinoline interfere by increasing the fluorescence and by removing the reagent. The fluorescence is decreased slightly in solutions containing other ions, and calibration curves should be constructed with the same amount of extraneous salts present as are to be expected in the unknowns. The errors in most cases are slight. Calibration curves for various amounts of magnesium ion are shown in Figure 4. Except for the relatively large error when magnesium is first introduced into the sample, slight variations in the total amount of magnesium present cause no appreciable error.

In Table I are shown the variations in the apparent amount of zinc present caused by adding 1 mg. of extraneous salt. The values were calculated by averaging the errors shown when 25 to 500 mg. or more of added salts were present and the amount of zinc was 0.21 mg. In most cases 50 mg. of material will cause an error of 0.02 mg. of zinc or less. Not more than about 3 mg. of fluoride ion should be present.



Table I. Influence of Extraneous Salts

Salt Added	Error, Mg. of Zn per Mg. of Added Salt	Range of Amounts of Added Salt Investigated, Mg.
$(\text{NH}_4)_2\text{C}_4\text{H}_4\text{O}_6$	0.0005	50-500
NaCl	0.00004	360-1800
$\text{KH}_2\text{PO}_4$	0.0002	27-135
KI	0.00015	50-200
$\text{KNO}_3$	0.00007	50-500
$\text{MgSO}_4$	0.0008	27-108
$\text{BaCl}_2$	0.0001	200
$\text{Ca}(\text{NO}_3)_2$	0.0003	70-700
HF	0.0034	2-20
$\text{Na}_2\text{SiO}_3$	0.0004	10-100

Table II. Typical Analyses

Zinc Taken Mg.	Zinc Found Mg.	Error Mg.
0.082	0.066	-0.016
0.164	0.153	-0.011
0.247	0.256	+0.009
0.369	0.378	+0.009
0.451	0.450	-0.001
0.492	0.481	-0.011

## INFLUENCE OF OTHER FACTORS

The amount of gum arabic present influences the intensity of fluorescence. The fluorescence is more intense when no gum arabic is present but the stability of the turbidities is less. Some turbidities showed only a 2% decrease in fluorescence between 2 and 8 minutes, while by the end of 8 minutes others decreased to one tenth of their value at 2 minutes. When 2 ml. of 2% gum arabic solution are present the fluorescence remains constant for 25 minutes or longer. The addition of 4 ml. of gum arabic instead of 2 ml. causes a decrease in fluorescence corresponding to an error of only 0.005 mg. of zinc.

The addition of only 1 ml. of ammonium acetate results in a pH value of 5.3 which is slightly low for complete precipitation of such small amounts of zinc. The decrease in per cent fluorescence corresponds to an error of about 0.025 mg. of zinc. When 10 ml. of 2 N ammonium acetate were used instead of 5 ml. no appreciable variation in the intensity of fluorescence could be noted.

The effect of adding the ammonium acetate last rather than the 8-hydroxyquinoline was investigated. Only a slight change in fluorescence was noted corresponding to an error of 0.007 mg. of zinc.

## DISCUSSION

In spite of the above observations, it is strongly recommended that all details of procedure, once established, be carefully followed. The calibration curves should be prepared under conditions as nearly like that of the unknowns as is possible. For more exact work, a final comparison with a standard solution containing the determined amount of zinc should be made.

The method is very rapid. A determination can be carried out, once the calibration curves have been prepared, in about 3 minutes. The accuracy is about 0.01 to 0.02 mg. of zinc. Results of several typical analyses are given in Table II.

Zinc in 1-gram samples of the Bureau of Standards aluminum alloy 86b was separated according to the method outlined by Churchill and Bridges (12). The zinc was finally made up to 250-ml. volume and a 5-ml. aliquot portion taken for analysis. Results of several determinations gave 1.4% of zinc. The accepted value is 1.51% of zinc.

Attempts to produce reproducible turbidities with 8-hydroxyquinoline and magnesium and aluminum ions have not been successful, as yet.

A dichlorofluorescein solution may be used for resetting the instrument at the same reading and is quite stable. The method

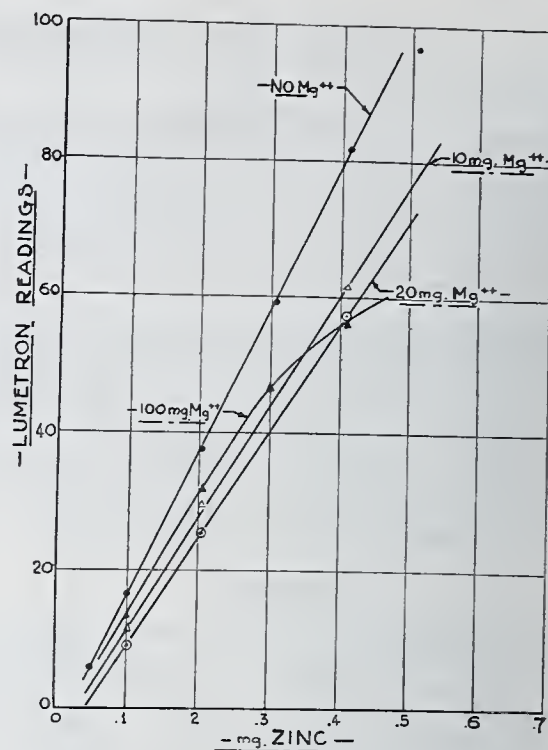


Figure 4. Influence of Magnesium

is reproducible. The results in Table II were obtained 9 days after the calibration curve was constructed.

## ACKNOWLEDGMENT

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## Cabinet for Boiler Feedwater Testing

The Testmaster cabinet, developed by TruTest Laboratories, Jefferson Bldg., Philadelphia 7, Pa., is an all-metal adjustable cabinet devised to accommodate tests for hardness, alkalinity, chloride, phosphate, pH, sulfite, and dissolved oxygen. Equipped with glareless fluorescent lighting, it presents a flexible and rapid means for testing boiler and feedwater samples to regulate treatment dosage and blow-down requirements.



# Detection of Bismuth by Means of Brucine Citrate

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A SURVEY of the literature on methods for the detection of bismuth disclosed several tests that indicated promise either in their present form or with slight modifications. One of the most satisfactory reagents for bismuth is thiourea, which has been considered by a number of investigators (1, 4, 5, 12). This reagent provides a highly selective test for bismuth, but is seriously handicapped by its lack of sensitivity. The use of various organic bases in conjunction with potassium iodide has been utilized in a number of procedures. Antipyrine (7), quinoline (6), cinchonine (3), 2-aminopyridine (11), 2-methylbenzothiazole (9), and numerous other bases have been applied with varying degrees of success. Reichard (10) reported that bismuth chloride reacts with brucine to form a red color. Moser (8) noted the same effect, but claimed that the reaction was uncertain.

The reported tests were carefully considered. Determination of sensitivities and investigations of interferences indicated that the reagents reported in the literature were generally unsatisfactory. It was, therefore, deemed advisable to undertake the development of new reagents. Different substituted thiourea compounds were investigated, but none was found to possess distinct advantages over the use of the parent compound itself.

A second field of investigation, the bismuth iodide-organic base complexes, was next studied. The reactions in this case seem to involve either the formation of normal salts with the bismuth iodide complex ion, or the formation of double salts. Several organic bases gave promise when used in this manner, but brucine was particularly satisfactory. It was noted that brucine was much more soluble in hot citric acid solution than in water. Other solvents that dissolved large amounts of brucine were ethyl alcohol, chloroform, acetic acid, and hydrochloric acid. None of these gave analytical characteristics comparable to those obtained with citric acid, although Korenman (7) had noted the very satisfactory sensitivity of an acetic acid solution of brucine when used in conjunction with potassium iodide to detect antimony, mercury, and bismuth. His studies, however, did not consider possible interferences, and the reactions were merely recorded, without further recommendations concerning the application of the reactions to test procedures. When brucine citrate was added to a solution containing bismuth and an alkali iodide was added, a yellowish orange precipitate was formed which changed after about a minute to an intense brick-red precipitate. The reaction was found to be applicable in any acid solution.

## METHOD OF STUDY

The determination of limiting concentration and limit of identification was performed in accordance with the procedures described by Feigl (2). Interference studies followed the general procedure discussed by West (13), except that the concentration of the bismuth solution was 0.1% while the concentration of the ions studied for interfering effects was 10%. A final check on interferences was made with more dilute solutions (0.01% bismuth to 1.0% interfering ion), so as to determine the reliability of the tests concerned near the sensitivity limit.

The ions investigated in the interference studies are listed below in their more common forms. It is realized that in

many instances the ions concerned are present as complexes, but where the structure of such complexes may be in doubt, only the valence of the central atom is indicated.

Li<sup>+</sup>, Na<sup>+</sup>, K<sup>+</sup>, Cu<sup>++</sup>, Rb<sup>+</sup>, Ag<sup>+</sup>, Cs<sup>+</sup>, Au<sup>+++</sup>, Be<sup>++</sup>, Mg<sup>++</sup>, Ca<sup>++</sup>, Zn<sup>++</sup>, Sr<sup>++</sup>, Cd<sup>++</sup>, Ba<sup>++</sup>, Hg<sup>+</sup>, Hg<sup>++</sup>, BO<sub>2</sub><sup>-</sup>, B<sub>4</sub>O<sub>7</sub><sup>-</sup>, Al<sup>+++</sup>, Sc<sup>+++</sup>, Ga<sup>+++</sup>, Y<sup>+++</sup>, In<sup>+++</sup>, La<sup>+++</sup>, Ce<sup>+++</sup>, Ti<sup>+</sup>, CO<sub>3</sub><sup>-</sup>, SiO<sub>3</sub><sup>-</sup>, Ti<sup>+++</sup>, Zr<sup>+++</sup>, Sn<sup>++</sup>, Sn<sup>+++</sup>, Pb<sup>++</sup>, Th<sup>++++</sup>, NH<sub>4</sub><sup>+</sup>, NO<sub>2</sub><sup>-</sup>, NO<sub>3</sub><sup>-</sup>, HPO<sub>4</sub><sup>-</sup>, P<sub>4</sub>O<sub>13</sub><sup>-</sup>, P<sub>2</sub>O<sub>7</sub><sup>-</sup>, PO<sub>3</sub><sup>-</sup>, VO<sub>3</sub><sup>-</sup>, HAsO<sub>3</sub><sup>-</sup>, HAsO<sub>4</sub><sup>-</sup>, Sb<sup>+++</sup>, Sb<sup>++++</sup>, S<sub>2</sub>O<sub>3</sub><sup>-</sup>, SO<sub>3</sub><sup>-</sup>, SO<sub>4</sub><sup>-</sup>, Cr<sup>+++</sup>, Cr<sub>2</sub>O<sub>7</sub><sup>-</sup>, SeO<sub>3</sub><sup>-</sup>, SeO<sub>4</sub><sup>-</sup>, MoO<sub>4</sub><sup>-</sup>, TeO<sub>3</sub><sup>-</sup>, TeO<sub>4</sub><sup>-</sup>, WO<sub>4</sub><sup>-</sup>, UO<sub>2</sub><sup>++</sup>, UO<sub>4</sub><sup>-</sup>, F<sup>-</sup>, Cl<sup>-</sup>, ClO<sub>3</sub><sup>-</sup>, ClO<sub>4</sub><sup>-</sup>, Mn<sup>++</sup>, MnO<sub>4</sub><sup>-</sup>, Br<sup>-</sup>, BrO<sub>3</sub><sup>-</sup>, I<sup>-</sup>, IO<sub>3</sub><sup>-</sup>, ReO<sub>4</sub><sup>-</sup>, Fe<sup>++</sup>, Fe<sup>+++</sup>, Co<sup>++</sup>, Co<sup>+++</sup>, Ni<sup>++</sup>, Ru<sup>+++</sup>, Rh<sup>+++</sup>, Pd<sup>++</sup>, Os<sup>++++</sup>, Ir<sup>+++</sup>, Pt<sup>+++</sup>, CN<sup>-</sup>, Fe(CN)<sub>6</sub><sup>-</sup>, Fe(CN)<sub>6</sub><sup>---</sup>, CNS<sup>-</sup>, acetate, oxalate, tartrate, aniline, pyridine

Table I. General Comparison of Brucine Citrate, Thiourea, Cinchonine, Antipyrine, 2-Methylbenzothiazole, and 2-Aminopyridine Tests for Bismuth

Test	Sensitivity	Interferences	
Brucine citrate	LI = 0.3γ LC = 1:100,000	Positive. Negative.	None Pb <sup>++</sup> , Hg <sup>+</sup> , Hg <sup>++</sup> , Ag <sup>+</sup> , Cu <sup>++</sup> , and Cd <sup>++</sup> (prevent full development of red color)
		Masking.	Tl <sup>+</sup> (yellow upon addition of KI), TeO <sub>3</sub> <sup>-</sup> (brown precipitate), Pd <sup>++</sup> (brown color), Hg <sup>+</sup> (black precipitate)
Thiourea	LI = 1.5γ LC = 1:100,000	Positive. Negative. Masking.	Sb <sup>+++</sup> , Pd <sup>++</sup> , VO <sub>3</sub> <sup>-</sup> , TeO <sub>3</sub> <sup>-</sup> None Hg <sup>+</sup> (black), SeO <sub>3</sub> <sup>-</sup> (red), SeO <sub>4</sub> <sup>-</sup> (red), Os <sup>++++</sup> (brown to pink), and colored ions such as Cr <sup>+++</sup> , Cr <sub>2</sub> O <sub>7</sub> <sup>-</sup> , UO <sub>4</sub> <sup>-</sup> , MnO <sub>4</sub> <sup>-</sup> , Rh <sup>+++</sup> , Pt <sup>+++</sup>
Cinchonine	LI = 0.3γ LC = 1:80,000	Positive. Negative. Masking.	Sb <sup>+++</sup> , Sb <sup>++++</sup> , Sn <sup>+++</sup> Pb <sup>++</sup> Ba <sup>++</sup> , Sn <sup>++</sup> Cu <sup>++</sup> (brown), Ag <sup>+</sup> (gray-brown), Au <sup>+++</sup> (black), Hg <sup>+</sup> and Hg <sup>++</sup> (black), Tl <sup>+</sup> (yellow), Cr <sup>+++</sup> and Cr <sub>2</sub> O <sub>7</sub> <sup>-</sup> (green), SeO <sub>3</sub> <sup>-</sup> and SeO <sub>4</sub> <sup>-</sup> (brown), TeO <sub>3</sub> <sup>-</sup> and TeO <sub>4</sub> <sup>-</sup> (black), Fe <sup>++</sup> and Fe <sup>+++</sup> (brown), Pd <sup>++</sup> (brown to black), and colored ions such as UO <sub>2</sub> <sup>++</sup> , UO <sub>4</sub> <sup>-</sup>
Antipyrine	LI = 0.3γ LC = 1:40,000	Positive. Negative. Masking.	Cd <sup>++</sup> , Tl <sup>+</sup> , Pb <sup>++</sup> , Sb <sup>++++</sup> Sn <sup>++</sup> , C <sub>2</sub> O <sub>4</sub> <sup>-</sup> Cu <sup>++</sup> (brown), Au <sup>+++</sup> (brown), Hg <sup>+</sup> (black), Cr <sup>+++</sup> and Cr <sub>2</sub> O <sub>7</sub> <sup>-</sup> (green), SeO <sub>3</sub> <sup>-</sup> and SeO <sub>4</sub> <sup>-</sup> (brown), TeO <sub>3</sub> <sup>-</sup> and TeO <sub>4</sub> <sup>-</sup> (black), Fe <sup>++</sup> and Fe <sup>+++</sup> (brown), Rh <sup>+++</sup> (red-brown), Pd <sup>++</sup> (brown), Os <sup>++++</sup> (blue to orange gray), and colored ions such as Co <sup>++</sup> , Ni <sup>++</sup> , Ir <sup>+++</sup>
2-Methylbenzothiazole	LI = 0.3γ LC = 1:40,000	Positive. Negative. Masking.	Tl <sup>+</sup> , Pb <sup>++</sup> , Sb <sup>+++</sup> , Sb <sup>++++</sup> Sr <sup>++</sup> , Ba <sup>++</sup> , NO <sub>2</sub> <sup>-</sup> , HPO <sub>4</sub> <sup>-</sup> , CN <sup>-</sup> , tartrate, citrate Cu <sup>++</sup> (brown), Au <sup>+++</sup> (brown), Hg <sup>+</sup> (black), Hg <sup>++</sup> (brown), Cr <sup>+++</sup> (green), Cr <sub>2</sub> O <sub>7</sub> <sup>-</sup> (green), TeO <sub>3</sub> <sup>-</sup> (black), Fe <sup>++</sup> (brown), Fe <sup>+++</sup> (brown), Pd <sup>++</sup> (brown), and colored ions such as UO <sub>2</sub> <sup>++</sup> , UO <sub>4</sub> <sup>-</sup> , Ru <sup>+++</sup> , Rh <sup>+++</sup>
2-Aminopyridine	LI = 10.0γ LC = 1:10,000	Positive. Negative. Masking.	Be <sup>++</sup> , Al <sup>+++</sup> , Ga <sup>+++</sup> , Y <sup>+++</sup> , Zr <sup>+++</sup> , Th <sup>++++</sup> , Sb <sup>+++</sup> , Sb <sup>++++</sup> Sr <sup>++</sup> , Ba <sup>++</sup> , Pb <sup>++</sup> , NO <sub>2</sub> <sup>-</sup> , HAsO <sub>3</sub> <sup>-</sup> , CN <sup>-</sup> , C <sub>2</sub> O <sub>4</sub> <sup>-</sup> Cu <sup>++</sup> (brown), Ag <sup>+</sup> (yellow), Au <sup>+++</sup> (brown), Hg <sup>+</sup> (black), Hg <sup>++</sup> (black), TeO <sub>3</sub> <sup>-</sup> (black), Fe <sup>++</sup> (yellow), Fe <sup>+++</sup> (yellow), and colored ions such as Cr <sup>+++</sup> , Cr <sub>2</sub> O <sub>7</sub> <sup>-</sup> , UO <sub>2</sub> <sup>++</sup> , UO <sub>4</sub> <sup>-</sup>



## REAGENTS

Brucine citrate. Dissolve 100 grams of citric acid in 100 ml. of water, add 12 grams of brucine and heat until solution is complete.

Borate inhibitor. Mix equal volumes of 1 M boric acid and 1 M sodium hydroxide.

Sodium bisulfite, saturated aqueous solution.

Potassium iodide, 20% aqueous solution.

## PROCEDURE

On a spot plate place one drop of the solution to be tested, and to it add one drop each of borate inhibitor, sodium bisulfite, brucine citrate, and potassium iodide. In the presence of bismuth a brick-red precipitate forms.

## DISCUSSION

The brucine citrate reaction, when used as a spot test, has a limit of identification of 0.3 microgram of bismuth at a limiting concentration of 1 part in 100,000. No positive interferences were found. Cadmium, mercury, copper, silver, and lead interfered, inasmuch as they prevented the full development of the red test color and reduced the sensitivity of the test. However, in their presence, a deep orange precipitate was formed which still permitted the absolute identification of the bismuth at a somewhat lessened sensitivity. The brucine citrate solution is very stable, one solution being in use in these laboratories for approximately one year without any change in appearance or reactions. Table I gives a comparison of some of the more important analytical characteristics of the brucine citrate, thiourea, cinchonine, antipyrine, 2-aminopyridine, and 2-methylbenzothiazole tests.

The test procedure followed in the case of thiourea was to add one drop each of 0.1 N nitric acid and 5% thiourea to a drop of the solution to be tested. For the cinchonine, antipyrine, 2-aminopyridine, and 2-methylbenzothiazole tests, a drop of

saturated sodium bisulfite was added to a slightly acid drop of the solution to be tested, followed by a drop of a 0.1% aqueous or alcoholic solution of the reagent and a drop of 20% potassium iodide. In the case of the four latter reagents many uncertain tests were obtained, owing to the liberation of iodine or the formation of yellow iodide precipitates or complex ions. Such reactions may mask the test for bismuth, or in many cases, the yellow colors formed may be confused with a true test for bismuth. The use of bisulfites does not completely obviate these difficulties.

For spot-test procedures, without prior separation of the bismuth, either the brucine citrate or the thiourea tests are satisfactory. The brucine citrate procedure has an advantage in both sensitivity and selectivity.

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## Microdetermination of Calcium

### By Titration of the Oxalate with Ammonium Hexanitratocerate

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THE limitations of potassium permanganate for microtitrations led several workers to substitute ceric sulfate (3, 5, 7, 8) and ceric ammonium sulfate (6) as oxidizing agents for the titration of oxalate. Although these two reagents form more stable and more easily prepared solutions than potassium permanganate, they still are not entirely satisfactory. These solutions do not always give either a sharp or a clear end point with the available indicators. In addition, the direct cerate titrations have to be carried out in a hot oxalate solution. At about the same time that the above ceric salts were proposed, Ellis (2) reported the use of ammonium hexanitratocerate for the drop-scale titration of calcium oxalate. Smith suggested the use of this salt (13) and carried out a series of studies concerning its properties (1, 10, 11, 12). Kirk and Tompkins (4), however, imply that this reagent is not suitable for microtitration of oxalate.

The need of a precise method for the determination of small amounts of calcium led the authors first to the use of ceric sulfate. The disappointing results with this procedure prompted them to begin an investigation of ammonium hexanitratocerate.

## SOLUTIONS

0.01000 N SODIUM OXALATE, 0.6700 gram of carefully dried Sorensen's sodium oxalate dissolved in 1 liter of distilled water. This solution is stable if kept in a refrigerator with chloroform as

a preservative, or if made in 0.1 N perchloric acid (9). To 4 ml. of this standard, 0.5 ml. of 60% perchloric acid is added before titration.

0.05% SETOPALINE C, 50 mg. of Eimer and Amend's setopaline C added to 100 ml. of distilled water and warmed on a steam bath or electric hot plate. The dye is only slightly soluble and precipitates on cooling. Therefore, the solution is warmed just before use, and 6 drops of the warm indicator are added to the oxalate solution. Furthermore, when the indicator is added as a cold solution, it is oxidized immediately by the first few drops of the cerate solution rendering it ineffective.

0.01 N AMMONIUM HEXANITRATOCERATE, 6 grams of reagent grade ammonium hexanitratocerate dissolved in about 200 ml. of 1 N perchloric acid and diluted to 1 liter with the acid. The solution is kept in a black bottle in the dark.

WASH SOLUTION (cf. 6), 2 ml. of 28 to 29% ammonium hydroxide, 98 ml. of distilled water, 100 ml. of redistilled ether, and 100 ml. of redistilled ethyl alcohol mixed together. The use of this solution always gave very good settling of the calcium oxalate on centrifugation. The use of 2% ammonium hydroxide, on the other hand, always resulted in the loss of material due to creeping and floating.

## PROCEDURE

The sample containing the calcium is pipetted into a 15-ml. conical centrifuge tube, and 2 drops of methyl red indicator and 1 ml. of 4% ammonium oxalate are added. Ammonium hydroxide (1 N) is added from a dropper until the indicator just turns yellow, and then 1 N hydrochloric acid is added until the



Ammonium hexanitratocerate in 1 *N* perchloric acid is a very good reagent for the microdetermination of calcium by direct titration of the oxalate at room temperature. The indicator setopaline C gives an extremely sharp end point with this reagent. Certain precautions must be observed in the preparation and storage of the ammonium hexanitratocerate solution. The reagent must be dissolved and kept in 1 *N* perchloric acid in order to prevent the irreversible hydrolysis of the salt. It is decomposed by light in either clear or brown glass bottles. The solution generally decreases in normality more rapidly when prepared in increased concentrations of perchloric acid. The solution, however, is stable in a black bottle, in which the decrease in titer is only about 2.5% over a period of 90 days. This change may be further lessened by keeping the bottle in the dark. Finally, the calcium oxalate must be dissolved in at least 1 *N*, but not concentrated, perchloric acid.

Pink color reappears. The solutions are stirred with footed glass stirring rods which are removed and hung on a numbered rack. After one hour, the tubes are centrifuged, the supernatant fluid is poured off, and the tubes are inverted on paper or cloth towels to drain for 2 minutes. Then the rims are wiped dry, the stirring rods are placed in their respective tubes, 5 ml. of wash solution is pipetted down the sides of the tubes, and the calcium oxalate precipitate is dispersed by stirring. The tubes are centrifuged and the wash procedure repeated. After the second wash, the precipitate is dissolved in 4 ml. of 1 *N* perchloric acid and titration is carried out in the centrifuge tube at room temperature. The tube is held by a clamp attached to the buret stand and the buret is lowered into the tube, so that the tip is always above the surface of the solution. The solution is stirred by an up-and-down motion of a narrow glass rod slightly flattened at the end and bent at a 45° to 90° angle at a height just above the lip of the tube. The warm indicator (6 drops) is added and the reagent is delivered from a microburet graduated at 0.02 ml. until the indicator changes from yellow to salmon pink. At the end point this color will persist for only about 15 seconds, after which it changes to a bronze. The color change is extremely sharp and distinct.

#### PREPARATION OF AMMONIUM HEXANITRATOCERATE SOLUTION

In order to obtain a satisfactory solution of the reagent, the salt must be dissolved directly in 1 *N* perchloric acid or in water, followed by the immediate addition of sufficient perchloric acid to make a 1 *N* solution. The former method is preferred. In the latter procedure, if the perchloric acid is not added immediately after the salt is completely dissolved, a fine insoluble precipitate appears. This precipitate is probably formed by hydrolysis of the salt (1). Furthermore, if the solution is warmed even after the above precaution has been observed, a large amount of fine white precipitate appears with a marked loss in titer. This latter fact apparently was not recognized by Kirk and Tompkins (4), for they warmed their solutions on the steam bath for 24 hours and filtered. Solutions prepared in the amounts of ammonium hexanitratocerate, stated by these authors to give a 0.02 *N* solution, gave a 0.05512 *N* solution when the heating procedure was omitted.

As a further check on the effect of heat, two solutions, 0.02672 and 0.00880 *N*, were prepared in 1 *N* perchloric acid, and aliquots from each of these were placed on the steam bath for 24 hours, filtered, and restandardized with sodium oxalate solution. The stronger of the two solutions decreased in normality to less than 10% of the original value and the weaker to less than 70%. In addition, the end point was blurred by the formation of a fine white precipitate during the titration. A similar decrease in titer as a result of heating was observed by Smith and Getz (12).

#### STABILITY OF AMMONIUM HEXANITRATOCERATE SOLUTION

The reagent rapidly decreases in titer when exposed to diffuse daylight (Table I). The decrease apparently is due to photo-

chemical reactions, since darkness prevented the change. Brown bottles also protected the ammonium hexanitratocerate solutions, but only when the concentration of the perchloric was not greater than 1 *N*. Black painted bottles provided the greatest protection, especially when placed in the dark.

#### CONCENTRATION OF PERCHLORIC ACID IN OXALATE SOLUTION

When the concentration of the perchloric acid in the standard sodium oxalate solution was less than 1 *N*, a white cloudy precipitate invariably formed on titration. On the other hand, if the normality of the perchloric acid was greater than 1 *N*, no further increase in accuracy was observed. The titration with a standard 0.02113 *N* sodium oxalate solution of fourteen equal aliquot portions of ammonium hexanitratocerate solution varying from 1 to 4 *N* in perchloric acid gave a mean normality of 0.01831 with an average deviation of only  $\pm 0.00004$ .

#### SOLUTION OF CALCIUM OXALATE

If the moist precipitate of calcium oxalate was dissolved in 0.5 ml. of concentrated perchloric acid and then diluted with 4 ml. of water, the results always were low and inconsistent (Table II). On the other hand, if the precipitate was previously dried or was dissolved in 1 *N* perchloric acid, theoretical values were obtained. It appears that the concentrated perchloric acid is able to oxidize the moist precipitate.

The figures in Table II also indicate the degree of accuracy of the method. Samples containing as low as 0.5 mg. of calcium have given similarly satisfactory results.

#### PRECIPITATION OF CALCIUM OXALATE

In the other reported procedures for calcium there is a wide variance in the manner of precipitation of the calcium oxalate; therefore, time of precipitation and digestion were studied. Furthermore, some of the authors' unknown samples required considerable ammonium hydroxide to bring them to the proper pH. Therefore, known samples were made excessively acid by the addition of 0.1 to 0.3 ml. of 6 *N* hydrochloric acid, and neutralized. The results in Table III indicate that one hour is suf-

Table I. Effect of Light and Concentration of Perchloric Acid on Stability of Ammonium Hexanitratocerate Solutions

Bottle	Place of Storage	HClO <sub>4</sub> Normality	Duration, Days	(NH <sub>4</sub> ) <sub>2</sub> Ce(NO <sub>3</sub> ) <sub>6</sub> Normality	Decrease, %
Clear glass	On bench	1	25	0.02182	21.1
		1	124	0.01722	6.8
		1	124	0.05453	1.0
		1	124	0.01795	6.1
		2	124	0.01777	5.0
		3	124	0.01771	4.2
Brown glass	On bench	1	84	0.05434	5.8
		1	84	0.01769	5.8
		2	84	0.01744	14.9
		3	84	0.01742	13.1
Black painted	On bench	1	90	0.06830	2.3
		1	90	0.02253	2.4
		2	90	0.02260	2.1
		3	90	0.02238	6.5
Black painted	In locker	1	80	0.01340	0.0

Table II. Effect of Perchloric Acid on Calcium Oxalate

Series No.	Number of Analyses	HClO <sub>4</sub> Normality	Condition of CaC <sub>2</sub> O <sub>4</sub> Ppt.	Average Ca Found, Mg.	Average Deviation <sup>a</sup>
1	4	9	Moist	0.968	0.223
2	12	9	Dry	1.203	0.015
3	8	1	Moist	1.208	0.013
4	14	1	Dry	1.217	0.004

Calcium by macroanalysis, 1.217 mg.

<sup>a</sup> Of a single observation.



Table III. Precipitation of Calcium Oxalate under Different Conditions

Series No.	Number of Analyses	Temperature for $\text{CaC}_2\text{O}_4$ Pptn.	Hours of $\text{CaC}_2\text{O}_4$ Pptn.	Excess $\text{NH}_4\text{Cl}$	Average Ca Found, Mg.	Average Deviation
1	10	Room	1	0	1.208	0.011
2	4	Room	20	0	1.211	0.006
3	4	Boiling water bath	2	0	1.208	0.009
4	6	Boiling water bath	3	0	1.200	0.017
5	9	Room	1	+	1.209	0.008
6	6	Boiling water bath	1	+	1.201	0.006

Calcium by macroanalysis, 1.217 mg.

\* Of a single observation.

cient time for precipitation and that digestion in a water bath or excess ammonium chloride does not increase or decrease the accuracy.

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## Microdetermination of Nitrates by the Devarda Method

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Microquantities (0.05 milliequivalent) of nitrates can be determined with a precision and accuracy of 99.8% by reduction with Devarda's alloy, the ammonia liberated being absorbed in boric acid and titrated with 0.01 *N* hydrochloric acid to bromocresol green-methyl red end point. By following the procedure given below, a single determination can easily be run in 20 minutes. Nitrites and ammonia interfere, but interference of ammonia can be easily overcome. Special apparatus described is convenient, but not essential.

**M**ETHODS for the determination of small quantities of nitrates have, in general, been either undesirably time-consuming or rather limited in accuracy. The Devarda method, generally accepted as the most desirable for macrodeterminations (4), appeared to be best suited to a microadaptation (1, 5), its only apparent disadvantage lying in the time required for an analysis and the relative complexity of the apparatus. In the hope of eliminating these disadvantages, the work described below was undertaken. Highly satisfactory results were obtained, and more than 200 determinations by the method finally developed have shown no undesirable features.

### APPARATUS

The apparatus shown in Figure 1 was constructed to facilitate the use of the method in conjunction with certain other experiments. It is described here, because it is well suited to the method and very convenient to use. Where the construction of such special apparatus is impractical, as for only occasional determinations, a satisfactory substitute can be assembled from ordinary laboratory apparatus.

The reaction and distilling flask, *A*, is a modification of the Parnas-Wagner micro-Kjeldahl apparatus (2, 3), fitted with standard-taper joints, *a*, *c*, and an electrical heating coil, *e*. The heating coil provides a convenient means of boiling the contents of the flask, being unaffected by drafts and capable of accurate control by means of a rheostat. Since the coil has a negligible heat capacity, there is no lag when the current is turned on or off. Expanding air escaping from the stoppered inner tube, *d*, effectively prevents bumping, even when concentrated caustic is being boiled. Alternatively, where the volume of liquid is small, distillation can be accomplished by an outside source of steam entering through the inner tube at *a*.

The spray trap, *B*, and condenser, *C*, are a single unit, with standard-taper joints to fit the distilling and receiving flasks.

The spray trap is simply a tube containing a wad of glass wool, *f*. This trap is essential, since the reaction mixture evolves a large amount of fine alkaline spray. It is insulated by a silvered vacuum jacket to eliminate condensation as much as possible. (A steam jacket or electrical heating coil may be substituted for this jacket, as in the macromethod described by Scott, 4.) It is important that the inner tube of the trap be unconstricted at the ring seal, *g*, so that the glass wool may be readily replaced.

The standard taper joint, *i*, is primarily a matter of convenience, as well as insurance against breakage of the condensate delivery tip, *j*. When the receiver, *k*, is connected by means of this joint, one can be certain that the delivery tip is covered by a maximum depth of liquid, without danger of its pressing against the bottom of the flask. Contamination is also minimized, since only the small vent, *h*, is open to the atmosphere. The receiver, *k*, is a 125-ml. Erlenmeyer flask.

Incidentally, the experience of the writer has been that the entire apparatus may be safely supported by one clamp on flask *A*. The trap-condenser unit is sufficiently strong to allow suspension by joint *c* alone, thus simplifying assembly, and eliminating the danger of breakage through incorrect alignment of clamps.

### EXPERIMENTAL

c.p. potassium nitrate was recrystallized three times from double-distilled water, and dried for 3 hours at 110° C. and 3 hours at 200° C. (4). The product was assayed by the ferrous sulfate method, and, indirectly, by conversion to the chloride and weighing. Both methods gave a potassium nitrate content of 100%. Probable impurities were tested for, and found absent. Standard solutions were prepared from this salt by weighing and diluting to a definite volume. Fresh solutions were prepared every day.

The procedure followed in all tests was as follows: A definite volume of 0.01 *N* nitrate solution was pipetted into the reaction flask through joint *c*, after which a weighed quantity of Devarda's alloy was added. The inside of the joint was washed down with sufficient water to bring the total volume to 20 ml., and the spray trap and condenser were connected. The receiving flask, containing 10 ml. of 2% boric acid and 2 drops of bromocresol green-methyl red indicator (2), was connected to the condenser, 10 ml. of 20% sodium hydroxide were added through funnel *b*, and the funnel stopcock was immediately closed. The mixture was then heated by the electrical heating coil until the reaction proceeded vigorously, when the current was turned off. After a definite length of time, low heat was turned on, and the mixture boiled until foaming had subsided. The heat was then increased, and about 10 ml. were distilled over. The receiver was lowered, and the distillation continued for about 30 seconds, the delivery tip being washed with a stream of water. The distillate was then titrated to a colorless end point with 0.01 *N* hydrochloric acid from a 5-ml. microburet.



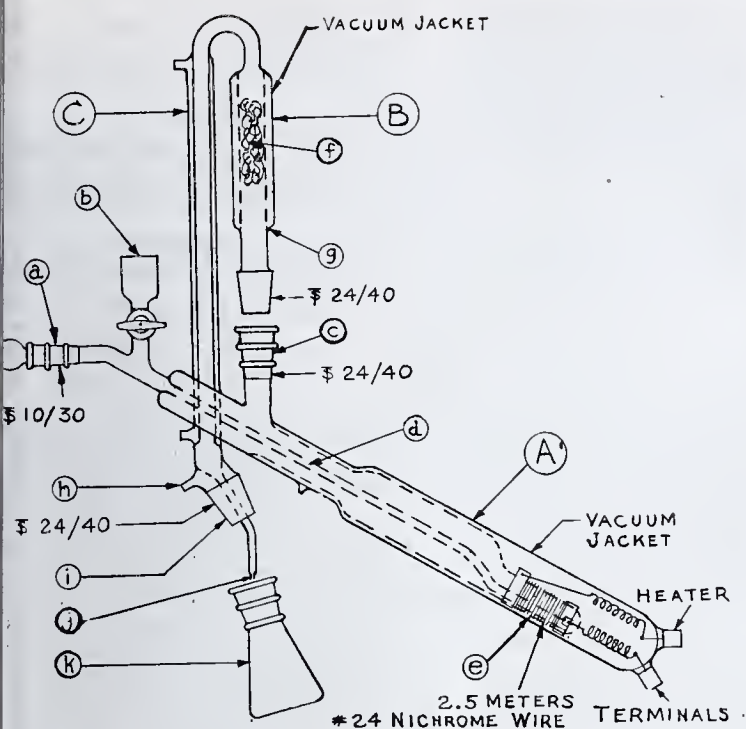


Figure 1. Distillation Apparatus

Table I. Effect of Reaction Time

Reaction Time Min.	Volume of 0.01153 N HCl Ml.	Weight of KNO <sub>3</sub>	
		Known Mg.	Found <sup>a</sup> Mg.
60	4.470	5.055	5.065
	4.460	5.055	5.053
	4.458	5.055	5.051
			Av. 5.056
30	4.462	5.055	5.056
	4.468	5.055	5.063
	4.462	5.055	5.056
			Av. 5.058
0	4.475	5.055	5.071
	4.465	5.055	5.059
	4.450	5.055	5.042
			Av. 5.057
15	0.128	Blank	0.149
	0.124	Blank	0.145
			Av. 0.147

<sup>a</sup> Final figures given in this and following tables are corrected for blank error. All titrations shown are original buret readings.

Considerable difficulty was at first encountered in obtaining consistent results, particularly from tests run on different days. The trouble was finally traced to the rubber tube connecting the microburet to the standard acid reservoir. Tests showed that 0.01 N hydrochloric acid standing in the rubber tube for 48 hours was reduced in concentration by 37%. A parallel test run on flexible plastic tubing showed a concentration reduction of only 0.9%. Accordingly, the rubber tube was replaced by the plastic, after which no further trouble was encountered.

It was decided first to determine the length of time actually required to effect a quantitative reduction of the nitrate by the process. In all tests, 5-ml. aliquots of 0.01 N potassium nitrate and 0.5-gram portions of Devarda's alloy were used, the reaction mixture being allowed to stand for varying lengths of time between initiation of the reaction and the preliminary boiling. The results of this experiment are given in Table I. Evidently, reaction time preliminary to boiling is not a factor in the accuracy of the method. For practical purposes, however, it is advisable to allow 5 to 10 minutes for the reaction to subside, since the mixture is apt to foam up into the spray trap if boiled immediately. For this reason, an interval of 10 minutes was allowed in all subsequent tests.

The quantity of Devarda's alloy necessary to complete the reaction was another factor open to question. Inasmuch as the alloy itself is the chief source of the blank error, it is desirable to

Table II. Effect of Weight of Devarda's Alloy

Weight of Alloy Gram	Volume of 0.01153 N HCl Ml.	Weight of KNO <sub>3</sub>		Recovery %
		Known Mg.	Found Mg.	
0.1	0.060	Blank	0.070	...
	0.060	Blank	0.070	...
	3.240	5.595	3.708	66.3
	3.210	5.595	3.673	65.7
0.2	4.684	5.595	5.353	95.7
	4.700	5.595	5.372	96.0
0.5	0.128	Blank	0.149	...
	0.124	Blank	0.145	...
	4.460	5.055	5.053	100.0
	4.485	5.055	5.081	100.5
1.0	0.275	Blank	0.321	...
	0.265	Blank	0.309	...
	4.600	5.055	5.049	99.9
	4.620	5.055	5.072	100.3

use as little as possible. Using an equal amount of standard potassium nitrate in each case, a series of tests was run with the weight of alloy as the variable (Table II). As was to be expected, a certain minimum weight (0.5 gram) of alloy was found necessary to produce stoichiometric results. Larger amounts merely increased the blank.

The two uncertain factors involved in the method having been determined, a series of analyses was run on samples of varying nitrate concentrations, in order to determine the method's range of accuracy. In addition to the purified potassium nitrate used in the previous experiments, samples of dried c.p. sodium nitrate were analyzed as an additional check (Tables III and IV).

## PRECISION AND ACCURACY OF METHOD

The deviations from the means of the analyses listed in Tables III and IV, expressed as weights rather than percentages, are

Table III. Effect of Size of Sample on Accuracy

Weight of KNO <sub>3</sub> Known Mg.	Volume of 0.01153 N HCl Ml.	Weight of KNO <sub>3</sub> Found Mg.	Deviation from Mean Mg.	Error %
Blank	0.128	0.149	...	...
	0.124	0.145	...	...
		Av. 0.147		
0.506	0.560	0.506	0.010	0.0
	0.540	0.483	0.013	4.5
	0.555	0.500	0.004	1.2
		Av. 0.496	0.009	1.8
1.011	0.995	1.013	0.002	0.2
	1.005	1.025	0.010	1.4
	0.990	1.007	0.008	0.4
		Av. 1.015	0.007	0.7
2.022	1.900	2.069	0.027	2.3
	1.860	2.022	0.020	0.0
	1.872	2.036	0.006	0.7
		Av. 2.042	0.018	0.9
5.055	4.470	5.065	0.011	0.2
	4.450	5.042	0.012	0.3
	4.465	5.059	0.005	0.1
	4.445	5.036	0.018	0.4
	4.465	5.059	0.005	0.1
	4.470	5.065	0.011	0.2
	4.458	5.051	0.003	0.1
		Av. 5.054	0.009	0.2

Table IV. Analyses of C.P. Sodium Nitrate

Weight of NaNO <sub>3</sub> Known Mg.	Volume of 0.01153 N HCl Ml.	Weight of NaNO <sub>3</sub> Found Mg.	Deviation from Mean Mg.	Error %
1.700	1.860	1.699	0.003	0.1
	1.855	1.694	0.008	0.4
	1.875	1.714	0.012	0.8
	1.862	1.701	0.001	0.1
		Av. 1.702	0.006	0.4
4.251	4.458	4.245	0.004	0.1
	4.465	4.252	0.003	0.0
	4.475	4.262	0.013	0.3
	4.450	4.238	0.011	0.3
	4.462	4.249	0.000	0.0
		Av. 4.249	0.006	0.1



fairly constant. They may be largely, if not entirely, attributed to unavoidable errors of measurement, since in only one case is the deviation greater than the equivalent of one drop in the titration.

The accuracy of the method is good: 99.8 to 99.9% for 0.05-milliequivalent samples. Considering the nature of the deviation from the mean, it is obvious that the accuracy of a single determination will decrease with smaller samples, and that the accuracy of the mean value of several determinations will decrease as the size of the sample approaches that of the blank. These facts are corroborated by the last columns of Tables III and IV.

#### INTERFERENCES

Certain types of nitrogen compounds may be expected to interfere with the method. Of these, ammonium and nitrite compounds are the most likely to be encountered. The former may readily be removed by adding alkali and boiling, before the addition of the Devarda's alloy. (If this procedure is followed, the mixture should be cooled thoroughly before adding the alloy.) Nitrites must be determined separately, and deducted from the total.

As opposed to the macromethod described by Scott, carbon dioxide shows no evidence of interference with this method.

#### OUTLINE OF PROCEDURE

**REAGENTS.** Devarda's Alloy, analyzed reagent.

Sodium Hydroxide, ammonia- and nitrate-free. Dissolve 200 grams of c.p. sodium hydroxide in water, and make up to 1 liter. Add about 1 gram of Devarda's alloy, and boil for about 10 minutes. Cool, replace any water boiled off, and store in a well-stoppered bottle (preferably Pyrex).

Boric Acid, 2% c.p. boric acid crystals in water.

Indicator, 10 ml. of 0.1% bromocresol green plus 2 ml. of 0.1% methyl red, in 95% ethanol.

**Standard Hydrochloric Acid.** Prepare approximately 0.01 *N* hydrochloric acid, and standardize against pure potassium nitrate by the method given. (Avoid contact of the standardized acid with rubber.)

**ANALYSIS.** Pipet 10 ml. of 2% boric acid into the receiving flask, and add 2 drops of bromocresol green-methyl red indicator. Place a fresh plug of glass wool in the spray trap. (Care should be taken to remove any fibers of glass wool from the ground joint.)

Place the sample in the distilling flask, and add 0.5 gram of Devarda's alloy. (Where a large number of determinations are to be made, measure the alloy by volume, using a cup made for the purpose.) Wash down with water, bringing the total volume to 20 to 25 ml. Connect the spray trap, condenser, and receiver. Add 10 ml. of 20% sodium hydroxide through the funnel, and close the stopcock.

Heat the mixture until it effervesces vigorously, and then let stand for at least 5 minutes. Again heat, gently, until foaming subsides. Then increase the heat, and distill about 10 ml. (If the spray trap is not vacuum-jacketed, electrical or steam heat should be turned on before beginning the distillation.)

Lower the receiver, and continue distillation for about 30 seconds, washing the delivery tip with water. Titrate the distillate with 0.01 *N* hydrochloric acid to a colorless end point.

It is advisable after each determination to replace the glass wool in the spray trap, and to rinse out the trap and condenser with water. The reaction flask should be cleaned in the following way: Rinse out most of the residue with water. Shake the flask with hydrochloric acid to dissolve the precipitated aluminum and zinc hydroxides and the residue of the alloy, and finally rinse again with water.

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## Microdetermination of Nitric Oxide in Gases

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Nitric oxide may be determined in olefin-free gases with an accuracy and precision of 99.0% by the method described. The gas is passed through an alkaline permanganate solution in a specially designed scrubber, after which the solution is analyzed by the micro-Devarda method. Gases containing unsaturated components may be analyzed with a modification of the procedure. If the olefin concentration is low, it is necessary only to increase the permanganate concentration of the absorbent. If it is high, the gas must first be scrubbed with sulfuric acid saturated with silver sulfate. Higher nitrogen oxides interfere. If present, they should be removed by scrubbing the gas with dilute alkali. The method may conveniently be used with nitric oxide concentrations down to 2 parts per million. A semiautomatic sampling apparatus for routine tests is described.

**B**ECAUSE of the insoluble and generally inert nature of nitric oxide, methods for its determination necessarily involve a preliminary oxidation to the more soluble nitrogen dioxide. Three oxidizing agents have been used in the most successful methods, all of which suffer from one or more disadvantages. Hydrogen peroxide, in acid, alkaline, and neutral solution, has been recommended by a number of investigators (1, 2, 3, 7, 15). However, the impurities it inevitably contains produce too high a blank to allow its use with low concentrations of nitric oxide (6). Fulweiler (4, 5, 6) has developed a generally satisfactory method of analysis, using oxygen and a catalyst mixed with the gas before

absorption. His apparatus, however, is rather complex, and a rather large correction factor must be applied. Oxygen alone can be used only with certain illuminating gases, which already contain catalysts (6, 13). Potassium permanganate has been used successfully by Shnidman and Yeaw (14), its only disadvantage lying in the fact that it is reduced by the olefins usually present in illuminating gases (6, 8). Since the gas to be analyzed by the writer contained negligible quantities of reducing compounds, permanganate appeared to be the oxidizing agent best suited to the purpose.

The nitrogen dioxide obtained from the oxidation of the nitric oxide has, in the past, almost invariably been determined colorimetrically, by absorption in a suitable reagent. Extreme sensitivity is thus obtained: the Griess-Ilosvay reagent is capable of detecting 0.1 microgram of nitric oxide as the nitrite (6). However, a rather low order of precision is obtained with all the methods reviewed, the variation in some cases exceeding the necessary correction factor. For this reason, the volumetric micro-Devarda method (10) was chosen for use with the method to be described. Alkaline permanganate was used as the oxidizing agent, so that a second scrubber to retain the nitrogen dioxide was unnecessary.

In order properly to develop and evaluate the method, it was necessary to test it against known mixtures of nitric oxide. As mentioned by Shnidman and Yeaw (14), the storage of previously prepared dilutions of nitric oxide is an exceedingly dubious proposition. For this reason, it was preferred to store pure nitric ox-



de, metering it directly into the gas stream during the absorption run. Water appeared to be the best confining liquid for the gas. Organic liquids were, of course, out of the question, and even mercury is attacked by the gas in time. However, nitric oxide is slowly decomposed to nitrogen in the presence of water (11). Consequently, the gas was assayed regularly with a gas buret, and renewed when the assay fell below 99%.

In order to avoid additional calculations and a possible source of error, the nitric oxide, which, of course, was saturated with water vapor, was passed through a desiccant before use.

#### EXPERIMENTAL APPARATUS

The apparatus used in the development of the method was, so far as possible, constructed entirely of Pyrex. Plastic tubing was used wherever flexibility was required, and full-length standard-taper joints were used wherever breaks in the line were necessary.

The layout of the apparatus was as follows: Nitrogen from a constant-pressure reservoir was led through a flowmeter to a mixing chamber, and then, by way of a 3-way stopcock, through either a capillary by-pass or the scrubber. Pure nitric oxide, stored over water in an all-glass chamber (Figure 1), was admitted through a phosphorus pentoxide jar to a calibrated capillary pipet of about 1-ml. capacity, from which it was displaced by mercury into the nitrogen stream in the mixing chamber. In order to prevent diffusion, the connection between the pipet and the mixing chamber was a fine capillary. A thermometer and manometer were provided, for use in calculation of volume corrections.

The nitric oxide content of the gas mixture was determined by the rate at which the mercury was admitted into the nitric oxide pipet. This was controlled by a leveling bulb lifted by a chain passing over a synchronous motor-driven sprocket. Thus, variations in the nitric oxide concentration could be obtained by varying the diameter of the sprocket.

#### EXPERIMENTAL PROCEDURE

At the start of a run, a capillary tube creating the same effective back pressure as the scrubber to be used was connected to the by-pass port of the 3-way stopcock. The nitrogen flow was started, and adjustments were made with the gas going through the by-pass.

The mercury in the nitric oxide delivery pipet was lowered below the T-connection to the nitric oxide reservoir, and the pipet was flushed with about 5 ml. of the gas. The synchronous motor feed was then started, the nitrogen-nitric oxide mixture being led through the by-pass until the mercury level reached the lower graduation of the pipet. Sufficient time elapsed in this period for the nitric oxide used in flushing the pipet to be swept out of the system.

The 3-way stopcock was then turned to pass the gas through the scrubber, and the time was noted. When the mercury reached the upper graduation of the pipet, the time was again noted, and the gas flow switched back to the by-pass.

The absorbent solution in the scrubber was then washed into the micro-Devarda reaction flask, and an amount of oxalic acid sufficient to reduce the excess permanganate to manganese dioxide was added. The solution was boiled down to about 20 ml., after which the micro-Devarda reaction and distillation were carried out in the usual way (10).

In the reduction of the permanganate, excess oxalic acid was required, in order to reduce the pH of the solution to a value where the reaction could occur. The oxalic acid remaining after the reduction had no effect upon the subsequent Devarda reaction. Incidentally, the final titration blank was not appreciably affected by any of these reagents.

#### DETERMINATION OF FACTORS INFLUENCING ACCURACY OF METHOD

A large number of preliminary runs were made, in the course of which several kinks in the apparatus were found and ironed out.

Table I. Effect of Gas Flow Rate

(Scrubber, 3 fritted-glass bubblers. Absorbent, 0.5%  $\text{KMnO}_4$ -0.5%  $\text{NaOH}$ .  $\text{NO}$  concentration, 27 micrograms per liter)

Flow Rate Ml./min.	Recovery %	Average %	Average Deviation %
300	94.9 97.4 95.9	96.1	0.9
600	84.7 86.4 86.7	85.9	0.8

The approximate optimum range of flow rate and absorbent concentration were determined, using as a scrubber a column of three fritted glass bubblers (9). A systematic study was then made of the four major variables affecting the accuracy of the method—i.e., the flow rate, the absorbent concentration, the nitric oxide concentration, and the type of scrubber.

The first runs were made with a concentration of 27 micrograms of nitric oxide per liter, since this was known to approximate the actual composition of the gas eventually to be analyzed. (Nitric oxide concentration is expressed in micrograms per liter, since that was the actual relationship measured. To convert approximately to parts per million by volume at 25° C. and 760 mm., multiply by 0.815.) An absorbent solution composed of 0.5% potassium permanganate and 0.5% sodium hydroxide was used, except when the effect of that concentration was being studied.

The first tests made were to determine the effect of the gas flow

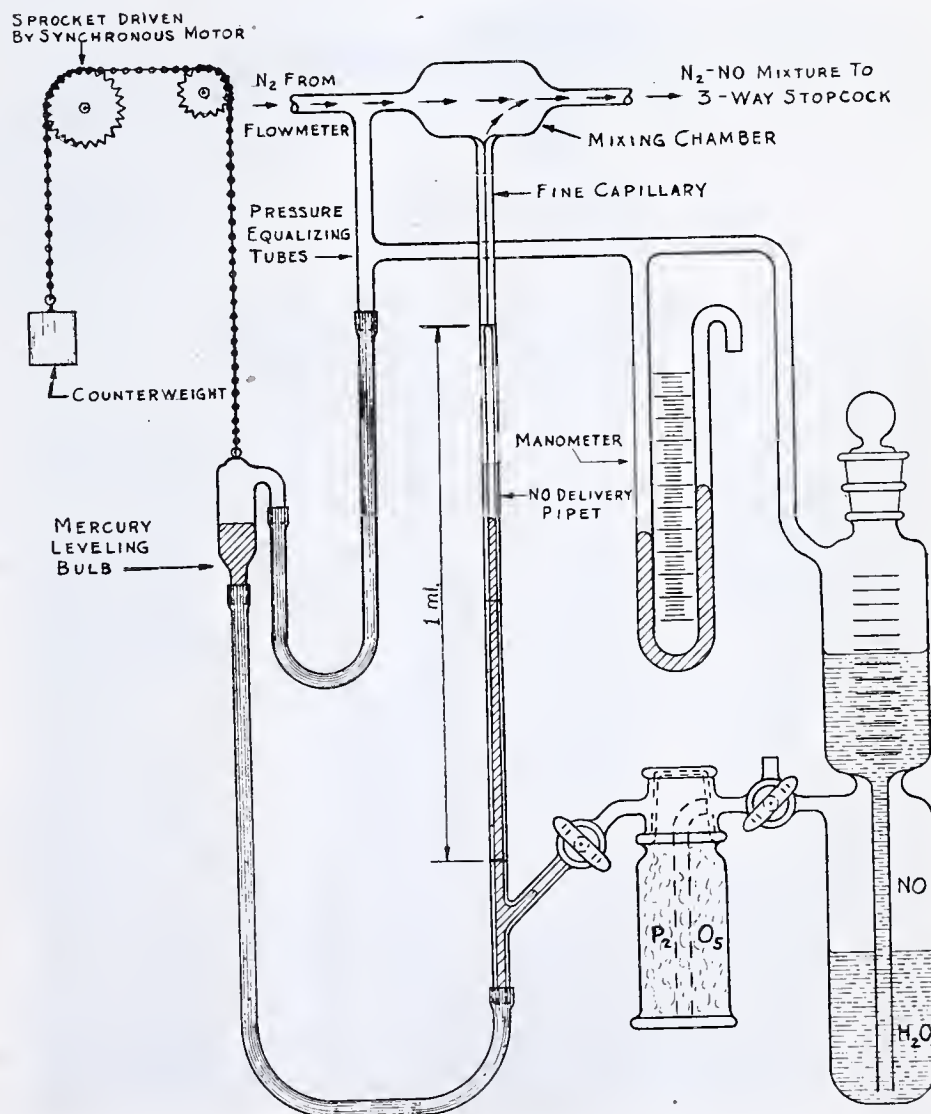


Figure 1. Nitric Oxide Feed Apparatus



rate, using a column of three fritted glass bubblers. The results are given in Table I.

Flow rates lower than 300 ml. per minute were not investigated, since the longer runs that would be required did not seem warranted by the 4% error to be corrected.

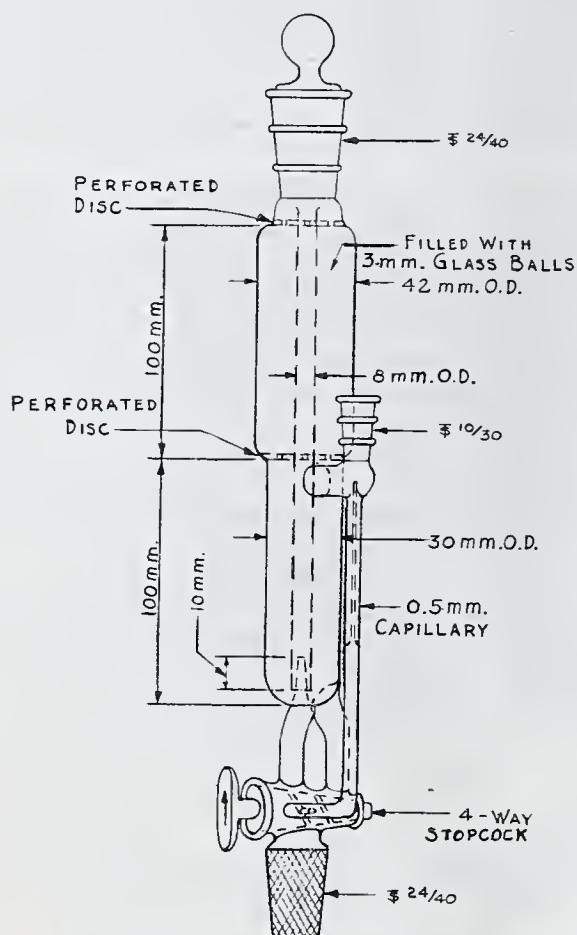


Figure 2. Modified Shaw Scrubber

The fritted-glass bubblers appeared to be entirely satisfactory, from the standpoint of efficiency. They had one disadvantage, however, in that they created a rather high back pressure: 72 mm. of mercury at 300 ml. per minute. For this reason, another type of scrubber, designed to create a low back pressure, was also tested. This scrubber (not illustrated) was a modification of that described by Shaw (12), in which the packed column section was enlarged to 200 mm. by 30 mm. in diameter. Its back pressure was 100 mm. of water at 300 ml. per minute. The results of flow rate tests with this scrubber (Table II), surprisingly enough, were almost identical with those obtained with the fritted-glass bubblers.

As compared with the fritted-glass bubblers, a rather large amount of water was required to wash the Shaw-type scrubber free of absorbent solution. However, this drawback was felt to be less disadvantageous than the high back pressure of the bubblers. Accordingly, all further tests were made with this scrubber. A flow rate of 300 ml. per minute was taken as a standard.

Tests were next made of the effect of absorbent concentration (Table III). A somewhat higher nitric oxide concentration was used, on the assumption that this would make more noticeable any falling off of efficiency at the lower absorbent concentrations. For simplicity's sake, equal amounts of potassium permanganate and sodium hydroxide were always used, the percentage

Table II. Effect of Gas Flow Rate

(Scrubber, modified Shaw scrubber. Absorbent, 0.5%  $\text{KMnO}_4$ -0.5%  $\text{NaOH}$ . NO concentration, 27 micrograms per liter)

Flow Rate Ml./min.	Recovery %	Average %	Average Deviation %
300	97.3 95.3 94.3 95.7	95.7	0.9
600	86.7 85.9 87.7	86.8	0.6

Table III. Effect of Absorbent Concentration

(Scrubber, modified Shaw scrubber. NO concentration, 55 micrograms per liter. Flow rate, 300 ml. per minute)

$\text{KMnO}_4$ - $\text{NaOH}$ Concentration %	Recovery %	Average %	Average Deviation %
0.5	97.0 96.5 97.1	96.9	0.2
0.2	97.3 95.3 96.0	96.2	0.7
0.1	92.4 90.0 90.6	91.0	0.9

indicated in the table referring to the concentration of each component.

The results of these tests demonstrate that maximum recovery is obtained as long as the concentration of the absorption reagents is greater than 0.2% potassium permanganate and 0.2% sodium

Table IV. Effect of Nitric Oxide Concentration

(Scrubber, modified Shaw scrubber. Absorbent, 0.5%  $\text{KMnO}_4$ -0.5%  $\text{NaOH}$ . Flow rate, 300 ml. per minute)

NO Concentration $\mu\text{g./l.}$	Recovery %	Average %	Average Deviation %
291	96.7	96.7	
58	97.0		
52	96.5		
54	97.1	96.9	0.2
26	97.3		
28	95.3		
27	94.3		
27	95.7	95.7	0.9
5.5	93.2		
5.7	93.1		
5.7	92.6	93.0	0.2

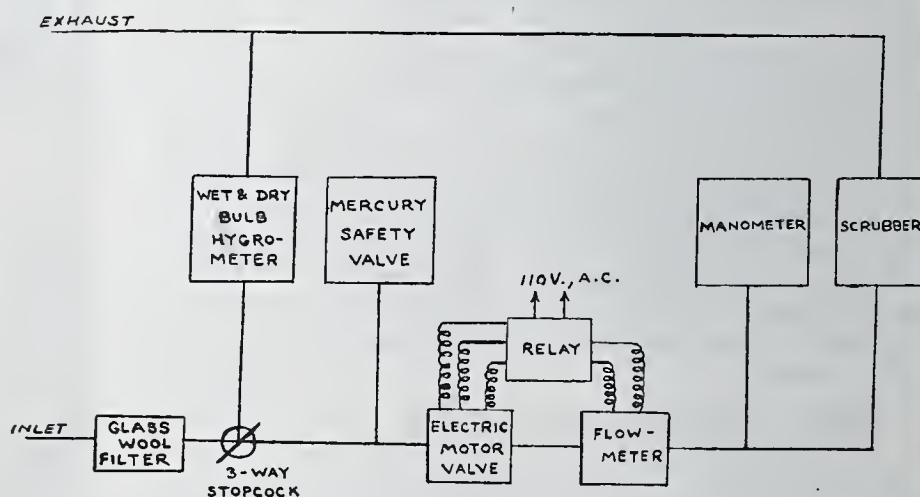


Figure 3. Flow Sheet for Gas-Absorption Apparatus



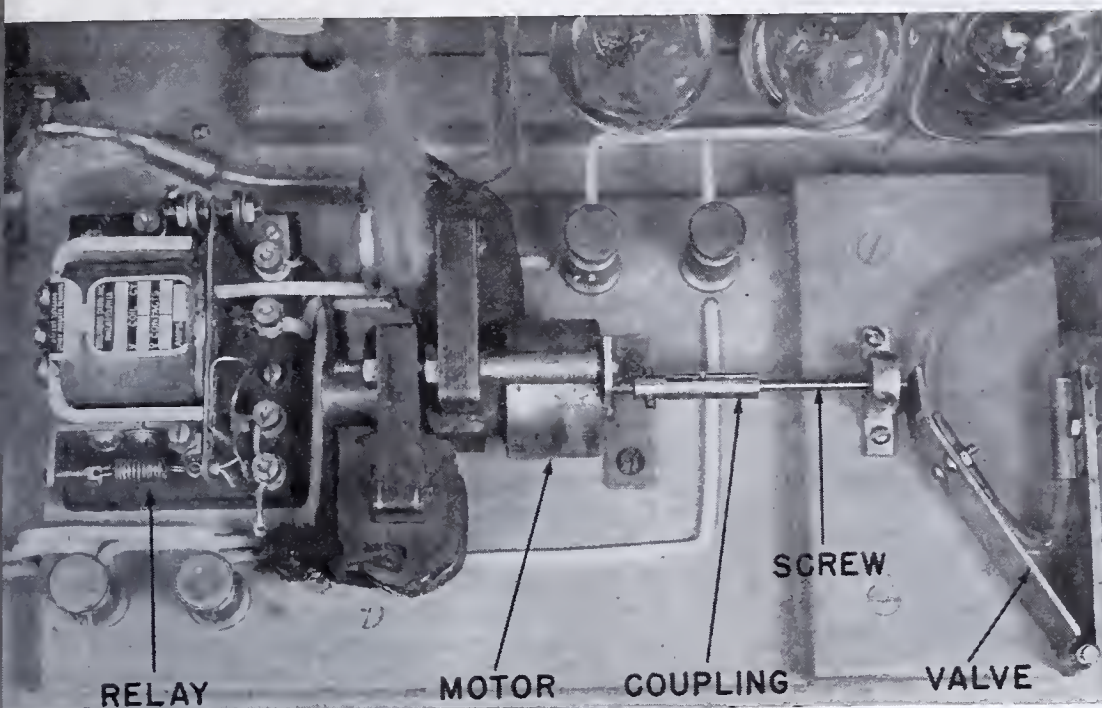


Figure 4. Motor-Driven Valve

hydroxide. To be on the safe side, a 0.5% potassium permanganate-0.5% sodium hydroxide solution was taken as the standard absorbent.

The optimum conditions of operation having been found, it remained only to determine the range of nitric oxide concentration over which the method was effective. A series of tests was accordingly made, in which the nitric oxide content of the gas was varied. The results of these tests are shown in Table IV.

These tests showed a definite falling off of scrubber efficiency at the lower nitric oxide concentrations. For this reason, as well as to make a more compact and convenient unit, the scrubber was redesigned. The final design is shown in Figure 2. The packed section of this scrubber has the same total volume as that of the first model, but is shorter and wider, thus producing a lower gas velocity for a given flow rate. The back pressure at 300 ml. per minute is 70 mm. of water.

Several other modifications were included in the new design, to simplify operation. Perforated glass disks sealed above and below the packed section keep the glass balls in place, and also reduce the probability of breakage of the inner tube. A capillary by-pass is incorporated directly into the unit, while a quarter turn of the special 4-way stopcock at the bottom directs the gas stream through either the by-pass or the scrubber. After a run, a quarter turn of the same stopcock opens the drain at the bottom of the unit. The standard-taper joint at the bottom serves to connect the scrubber to the gas line, or, while draining, to the reaction flask. In addition, it provides a convenient means of support for the apparatus, eliminating the need for a clamp.

The effect of nitric oxide concentration on the efficiency of the new scrubber was tested, as shown in Table V. For practical purposes, the efficiency of the redesigned scrubber was 100%. Since any loss of efficiency would appear at the lower nitric oxide concentrations, tests at high concentrations were not considered necessary.

connecting the pipet to the mixing chamber became a considerable factor. These two considerations easily account for the apparently poor showing of the scrubber. Inasmuch as the gas to be analyzed would seldom, if ever, contain such low nitric oxide

The low precision encountered in the tests at 2.6 micrograms of nitric oxide per liter requires some explanation. It was obviously desirable to test the scrubber at as low a nitric oxide concentration as possible, in order to detect any falling off of efficiency. However, in order to keep the duration of the test run within reasonable limits, it was necessary to use a smaller total volume of nitric oxide than usual. This was accomplished by raising the mercury in the nitric oxide pipet three-fourths of the pipet's length before starting the run. Because of the shorter distance then to be traveled by the mercury, errors of measurement were magnified. Furthermore, in view of the low velocity of the nitric oxide, diffusion of the gas contained in the capillary

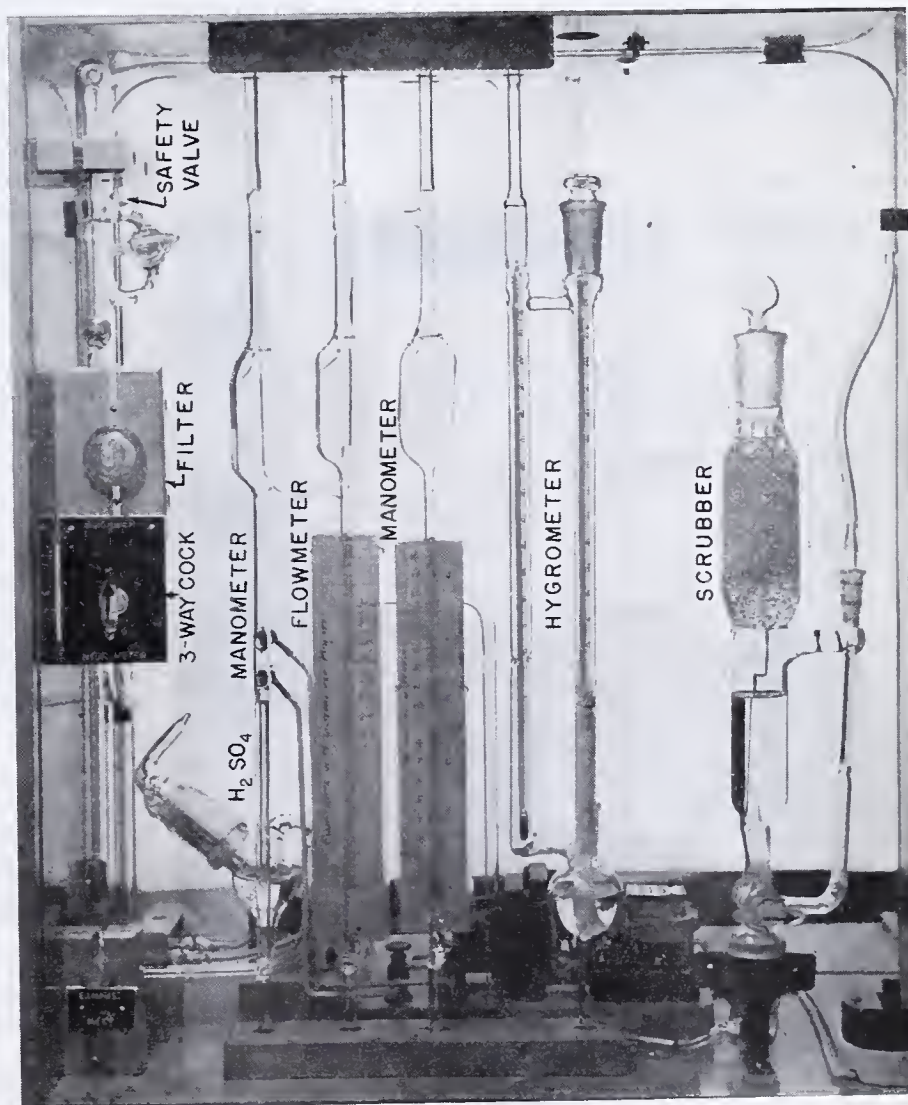


Figure 5. Gas-Sampling Apparatus, Front



Table V. Effect of Nitric Oxide Concentration

(Scrubber, modified Shaw scrubber No. 2. Absorbent, 0.5%  $\text{KMnO}_4$ -0.5%  $\text{NaOH}$ . Flow rate, 300 ml. per minute)

NO Concentration $\mu\text{g./l.}$	Recovery %	Average %	Average Deviation %
27	100.0		
26	98.9		
27	99.1	99.3	0.4
5.9	99.5		
5.6	100.0		
5.7	99.5	99.7	0.2
2.6	107		
2.6	98		
2.6	95	100	5

concentrations, it was not considered worth while to construct and calibrate a special pipet for this one test.

The average deviation figures given in the various tables actually represent the over-all precision of the test method, including the apparatus used to prepare the nitric oxide mixtures. The true precision of the analytical method is probably somewhat better than this.

#### SOURCES OF ERROR AND APPLICABILITY OF THE METHOD

Sources of error in the micro-Devarda procedure used in conjunction with this method have already been discussed (10). Obviously, all water and reagents used should be free of nitrates and ammonia. As mentioned above, the reduced absorption reagents have no effect upon the analysis. The only factor requiring special consideration in this application is the size of the sample. For the highest precision and accuracy, the sample should be large enough to require a titration of at least 1 ml.—i.e., not less than 0.25 mg. of nitric oxide. It is obvious, therefore, that accuracy must be balanced against convenience, where long runs would be required to analyze gases of low nitric oxide content.

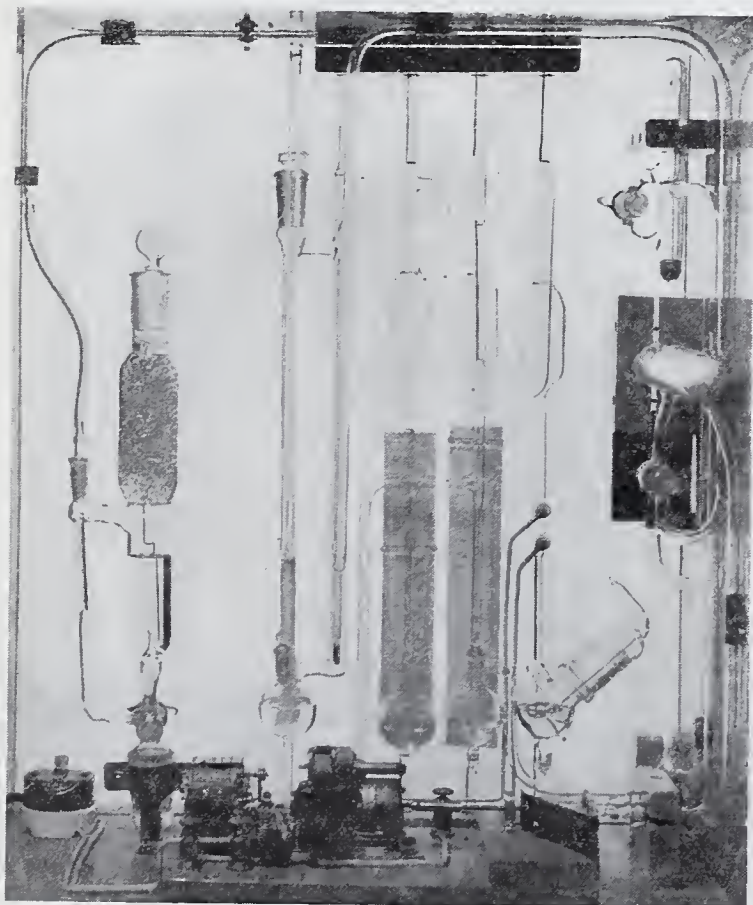


Figure 6. Gas-Sampling Apparatus, Rear

In this connection, it should be remembered that the method has not been checked against nitric oxide concentrations of less than 2.6 micrograms per liter. Judging by the behavior of the original modified Shaw scrubber, it appears possible that the efficiency of the improved scrubber might start to fall off at such a lower concentration. This possibility should be checked, if accurate determinations of very low nitric oxide concentrations are required.

Because of its convenience and compactness, a differential flowmeter of the capillary type is used by the writer for the measurement of the gas. (Where only a low gas pressure is available, a wet gas meter may be used to advantage.) The significance of an error in reading the flowmeter depends upon its design. If sufficient gas pressure is available, the differential may be made large enough to make reading errors negligible. The back pressure of the scrubber (70 mm. of water) introduces a small error in the flowmeter reading, which can be corrected. The variation in back pressure is too small to be significant. Timing the run is, of course, necessary, where a flowmeter is used. Errors from this source are ordinarily negligible.

Leaks in the apparatus obviously are to be avoided. Rubber tubing connections are undesirable from this standpoint, and also because of the fact that a certain amount of nitric oxide may be absorbed by the rubber. So far as possible, the apparatus should be all glass, with fused connections. Flexible connections are best made with plastic tubing.

Several of the first trials of the final scrubber design were spoiled by uneven wetting of the packing. This difficulty may be avoided by turning the charged scrubber over, and wetting all the balls with the absorbent solution. If the balls are uniformly wetted at the start, no appreciable channeling will occur during the run.

Excessive reduction of the permanganate absorbent by unsaturated compounds will, as indicated in Table III, give rise to a serious error. The effective potassium permanganate concentration must never fall below 0.2%. To this end, the initial concentration of the absorbent may be raised up to 5% potassium permanganate-5% sodium hydroxide, without otherwise affecting the analysis. If this means is insufficient to handle the reducing compounds, a preliminary scrubbing is necessary. Satisfactory results were obtained by the writer by scrubbing illuminating gas with sulfuric acid saturated with silver sulfate (16). The Shaw scrubber used for this purpose was similar to that illustrated in Figure 2, except that the liquid reservoir (lower section) had a capacity of 150 ml. About 18 liters of gas can be handled by 100 ml. of sulfuric acid-silver sulfate before olefins start to come through.

Nitrogen peroxide is likely to be found in any gas containing nitric oxide. Obviously, all the higher oxides will interfere. Unless known to be absent, they should be removed by a preliminary scrubbing of the gas with 0.5% sodium hydroxide. Incidentally, the higher oxides, as a group, may easily be determined by the present method, merely by substituting 0.5% sodium hydroxide for the 0.5% potassium permanganate-0.5% sodium hydroxide absorbent solution.

#### ROUTINE TEST APPARATUS

A semiautomatic, portable apparatus was constructed, for convenience in sampling gases at various locations. Its flow sheet is given in Figure 3. Gas entering the apparatus is freed of suspended matter by a glass wool filter, and then led by a 3-way stopcock through either a wet and dry bulb hygrometer or the flowmeter-scrubber system. (The hygrometer is used for determining the dew point of the gas. This measurement is required on the gas being analyzed by the writer, but is not related to the nitric oxide determination.)

A flow rate through the scrubber of  $300 \pm 2$  ml. per minute is automatically maintained by means of a motor-driven valve controlled by a differential capillary flowmeter. Dilute sulfuric acid in a manometer in parallel with the water-filled flowmeter manometer makes or breaks contact with fixed platinum con-



tacts. A small magnetic relay is thus operated, which, in turn, operates a reversible Telechron motor to close or open the valve. A bulb on an eccentric dipping into the well of the sulfuric acid manometer facilitates minor adjustments of the zero point.

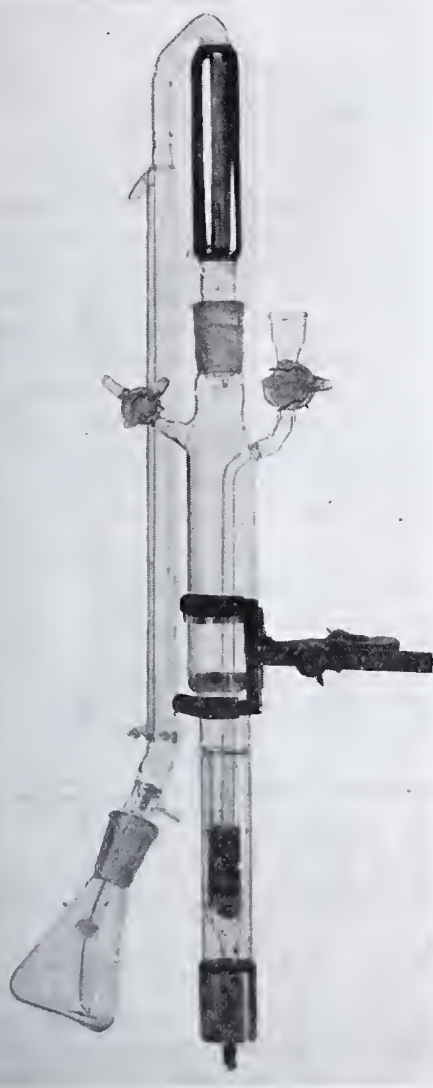


Figure 7. Micro-Devarda Apparatus

The motor-driven valve is shown in Figure 4. Its somewhat unique design is the result of experiments with various types of needle and pinch valves, none of which offered the sensitivity of control required for the purpose. In operation, a kink in a section of soft plastic tubing (3 mm. in inside diameter) is opened and closed by a screw, working against the hinge to which the tubing is clamped. A key and slot coupling connects the screw to the 1 r.p.m. synchronous motor.

The control mechanism operates best with an inlet gas pressure of between 2 and 10 cm. of mercury. Higher pressures are undesirable, in that they cause a wide fluctuation of the flow rate with the oscillation of the automatic valve. Variations in the gas pressure between the above limits have negligible effect, if they are not too rapid for the slow-moving valve to handle. The use of a pressure regulator on the gas line may be desirable, from this standpoint.

A mercury manometer, indicating the gas pressure on the apparatus, is provided with a trap, so that it serves as a safety valve as well. In addition, traps are provided on all the meters, to prevent loss of liquid in case of a sudden rise in pressure, or failure of the control.

A manometer is connected to the line between the flowmeter and the scrubber, indicating the back pressure on the flowmeter. It is calibrated in terms of the correction necessary to be applied to the flowmeter reading. Since the flowmeter was calibrated against a back pressure equal to that created by the scrubber, this correction ordinarily is zero.

Both the flowmeter and manometer are provided with capillary tubes to damp the minor oscillations caused by the bubbling of the gas through the scrubber. Reading of the meters is thus greatly facilitated.

The assembled sampling apparatus is shown in Figure 5 (front view) and Figure 6 (rear view). It is relatively compact and easily portable. The cabinet is provided with removable sliding doors, front and back.

The micro-Devarda flask, used in the analysis of the dissolved sample, has been somewhat simplified (Figure 7). The original flask (10) was entirely vacuum-jacketed, to prevent excessive condensation, when distilling with an outside source of steam. This feature is not required for the present application, resulting in a more easily constructed and compact apparatus. The electrical heating coil alone is vacuum-jacketed, chiefly for protection. Electrical connections are made through a standard 2-prong plug, cemented to the flask, to make a base similar to that of a radio tube. The leads are thus protected, and connection to an extension cord is simplified. A stopcock is provided on the left side of the flask, where suction may be applied to facilitate drainage of the scrubber into the flask. As the photograph shows, the flask is used in an upright position, making for a more compact distillation set up.

Operation of the apparatus is very simple. The gas inlet and exhaust lines are connected to the respective ports (lower left corner, Figure 5). The 3-way stopcock (left side, Figure 5) is turned to "Hygrometer", and the gas allowed to flow at a maximum rate until the wet-bulb reading becomes constant. This serves the double purpose of determining the humidity of the gas and flushing out the lines. Meanwhile, the scrubber is charged, and connected in place, its 4-way stopcock being set to the by-pass position.

The 3-way stopcock is then turned to "Scrubber", and the electricity is turned on. When the flowmeter reading settles down at 300 ml. per minute, the scrubber's 4-way stopcock is turned to pass the gas through the scrubber, and the time is noted. The back-pressure manometer reading is checked for any abnormality, such as might be caused by a leak or stoppage in the lines. (It normally shows only slight variation between or during runs.) No further attention is required until the end of the run. The 4-way stopcock is then returned to the by-pass position, the time is noted, and the gas and electricity are turned off.

The scrubber is placed in the standard-taper neck of the micro-Devarda flask, the stopcock turned to the drain position, and the absorbent washed into the flask with water. Ten milliliters of 6% oxalic acid are added, and the mixture is boiled down to about 20 ml. The use of a spray trap during the evaporation is desirable, any material trapped being washed back into the flask. The micro-Devarda procedure is followed from there in the usual way.

It is advisable to wash the scrubber promptly after its use, to prevent etching of the glass balls and freezing of the stopcock by any residual alkali. Hydrochloric acid followed by water serves this purpose, dissolving any deposited manganese dioxide as well.

#### CALCULATIONS.

$$\frac{(\text{Ml. of HCl} - \text{blank}) (\text{NF}) (30,000,000)}{(\text{Duration of run in minutes})(\text{flowmeter reading} + \text{correction, in ml. per min.})} = \text{micrograms of NO per liter}$$

$$(\text{Micrograms of NO per liter})(0.815) = \text{p.p.m. of NO by volume at } 25^{\circ} \text{ C. and 760 mm.}$$

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# NOTE ON ANALYTICAL PROCEDURES

## Ground Starch as an Indicator in Iodometry

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THE useful life of the solution of soluble starch used as a conventional indicator in iodometry may be prolonged with any one of a number of disinfectants. However, hydrolysis of the starch eventually renders the reagent unsuitable for use, and therefore it has been found advantageous in many cases to replace the solution with a dry preparation of ground starch.

The starch is prepared by a procedure similar to that recommended by Alsberg, Griffing, and Field (1) and by Schoch (3). A convenient quantity is suspended in about twice its weight of ethyl alcohol and ground in an efficient ball mill for at least 80 hours, at which time a microscopic examination will reveal few, if any, granules that have escaped disintegration. The ground starch is filtered off, dried, and then reground. The resulting preparation, which is almost completely soluble in cold water, is ready for use. An inexpensive salt or pepper shaker has been found to be a convenient container and dispenser for the starch. If desired, a solution of starch may be prepared without the use of heat by simply adding the ground starch to cold water.

Presumably any available starch may be used, but soluble starch is particularly satisfactory. The time required for grind-

ing will depend on various factors, including the particular mill used, the charge, the kind of starch, and the starch-alcohol ratio. The preparation may be kept indefinitely without charge.

Iodometric titrations are carried out in the usual manner, a small amount of the ground starch being added near the end point. Titrers obtained with this indicator were checked against those obtained with a solution of soluble starch prepared according to the directions of Lange (2). Aliquots of a potassium dichromate solution were titrated in the conventional manner with 0.01 N sodium thiosulfate. An average of five titers using a soluble starch solution gave a value of 32.40 ml.; with ground starch the same average of 32.40 ml. was obtained.

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## CORRESPONDENCE

### *o*-Cresol in Phenol

SIR: We have subjected the "cloud-point" method of Seaman, Norton, and Foley [*IND. ENG. CHEM., ANAL. ED.*, **15**, 159 (1943)] to brief examination and, while we found it an eminently practicable and useful procedure within the limits laid down, we also found that exact confirmation of results was not obtained. The root cause of the discrepancy was discovered in the figure observed for the "cloud-point temperature" of pure phenol and water when mixed in the prescribed proportion; this is quoted as 66.40° in the article, whereas we obtained a figure of 66.10° C.

The phenol used in our short series of experiments had a cloud point (Bell and Herty, Standardization of Tar Products Tests Committee procedure) of 41.0° C. and the *o*-cresol had a cloud point of 31.0° C. The results of our tests are given here.

Observed Cloud-Point Temperature ° C.	<i>o</i> -Cresol in Mixture with Phenol %	<i>o</i> -Cresol Calculated by Equations Given Below %
66.10	0.00	0.00
67.95	1.45	1.45
70.40	3.39	3.39
73.00	5.52	5.52

It was found necessary, in order to bring observed and calculated *o*-cresol contents into line, to modify the equations given in the article as follows:

For cloud-point temperatures up to 70.25° C.:

$$\% \text{ } o\text{-cresol} = \frac{\text{cloud point (° C.)} - 66.10}{1.273} \quad (1)$$

For cloud-point temperatures 70.25° to 73.5° C.:

$$\% \text{ } o\text{-cresol} = \frac{\text{cloud point (° C.)} - 66.25}{1.222} \quad (2)$$

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SIR: Regarding discrepancy between the cloud point of 66.40° C. for pure phenol and water which we reported [*IND. ENG. CHEM., ANAL. ED.*, **15**, 159 (1943)] and the figure which Kay and Haywood have obtained, 66.10° C.:

We have repeated some of the experiments and think that we have found the explanation for the discrepancy. Instead of one abrupt change in appearance, there are really two. We took the first one (at 66.40° C.) and evidently they must have taken the second one (at 66.10° C.). It is impossible to describe these points in words, but after one has observed both, one can always detect either point with good precision. Three of us have checked each other quite well at both points. Furthermore, there is a similar difference when determinations are made with phenol containing added *o*-cresol, although we have not done sufficient work to know whether the difference will remain constant or not over a range of concentrations. It may be that a variation in the magnitude of the differences with a variation in *o*-cresol concentration may explain the fact that the slopes of the lines calculated from the four determinations which were sent us differ from those which we reported (1.273 and 1.222 instead of 1.326 and 1.167, respectively).

It would seem likely that either point can be used, provided that the analyst constructs his curve on the basis of that point; but since we have been able consistently to get reproducible values with the point which we had chosen, it might be desirable to have the analyst look for that point, in which case he could use our equations, rather than to use the other point and have to repeat the large number of determinations which would be necessary to establish accurate equations.

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# AUTHOR INDEX

VOLUME 16—1944

- ACREE, FRED, JR. See Jacobson, Martin.
- AIKEN, W. H. See Doty, P. M.
- ALBANESE, A. A., WAGNER, D. L., FRANKSTON, J. E., AND IRBY, VIRGINIA. Amino Acid Analysis of Some Common Vegetables. Method for Carbohydrate-Free Extraction of Nitrogen from Fresh Vegetables..... 609
- ALLEN, C. C., AND DUCKWALL, H. W. Sulfuric Acid Extraction in Hydrocarbon Type Analysis. (Correction, 727)..... 558
- ALTHOUSE, P. M., AND TRIEBOLD, H. O. Physical Constants of Methyl Esters of Commonly Occurring Fatty Acids. Vapor Pressure..... 605
- AMOROSI, A. M. See McKinney, D. S.
- AMSTUTZ, E. D. See Fehnel, E. A.
- APPLEZWEIG, NORMAN. Large-Capacity Continuous Solids Extractor. 472
- APPLING, J. W. See Wise, L. E.
- ARCHIBALD, R. M. See Hamilton, P. B.
- ARNOLD, M. R. See Dunbar, R. E.
- ASHBURN, GILBERT, AND FRANK, R. L. Constant-Rate Dropping Funnel..... 418
- ASHLEY, S. E. Q. See Murray, W. M., JR.
- ATKIN, LAWRENCE, WILLIAMS, W. L., SCHULTZ, A. S., AND FREY, C. N. Yeast Microbiological Methods for Determination of Vitamins. Pantothenic Acid..... 67
- AUERBACH, M. E. Colorimetric Assay of Quaternary Ammonium Salts..... 739
- BAER, ERICH. Improved Distilling Flask..... 399
- BAIER, W. E. Adaptor for Angle Centrifuge Tests..... 193
- BAKER, IRVIN, MILLER, MARTIN, AND GIBBS, R. S. Colorimetric Microdetermination of Tin with Silicomolybdate..... 269
- BANDEMER, S. L., AND SCHAIBLE, P. J. Aid in Ashing Certain Materials. Determination of Iron. Study of *o*-Phenanthroline Method..... 417
- BANGERT, W. M. See Boericke, F. S.
- BARNES, R. B., WILLIAMS, VAN ZANDT, DAVIS, A. R., AND GIESECKE, PAUL. Physical Methods of Analysis of Synthetic and Natural Rubber. (Correction, 486)..... 9
- BARNES, R. H., RUSOFF, I. I., MILLER, E. S., AND BURR, G. O. Relationship between Unsaturation and Ultraviolet Absorption Spectra of Various Fats and Fatty Acids..... 385
- BARTHEL, W. F. Improved Vacuum Distilling Head..... 374
- AND LAForge, F. B. Determination of Carbon-Linked Methyl Groups..... 434
- See also Bowen, C. V.
- BARTON, C. J., AND PRUTTON, A. J. Photometric Method for Determination of Hemicellulose..... 429
- BATEN, W. D., AND DEWITT, C. C. Use of Discriminant Function in Comparison of Proximate Coal Analyses..... 32
- BATTISTA, O. A. Molecular Weight of Cellulose. Measurement of Average Degree of Polymerization..... 351
- BAYLEY, C. H. See Weatherburn, A. S.
- BECK, CLYDE. See Lang, O. W.
- BENJAMIN, HENRY. Fluorocolorimetric Determination of Blended Oils and Oil in Oil-Water Emulsions..... 331
- BENNE, E. J. Washing Selas Filtering Crucibles by Reverse Flow..... 277
- See also Comar, C. L.
- BERO, RUTH. See Gawton, Oscar.
- BERKOWITZ, DONALD AND BERNSTEIN, RUBIN. Analysis of Soap—Synthetic Detergent Mixtures in Bar Form..... 239
- BERNSTEIN, RUBIN. See preceding item.
- BESHOETOOR, A. W., GREENE, L. M., AND STENGER, V. A. Determining Phenols in Dilute Solutions. Notes on Gibbs Method... 694
- BROOS, B. S., AND ERICKSON, R. H. Determining Plasticizer Content of Cellulose Esters..... 93
- BIMMERMAN, H. G., AND KEEN, W. N. Use of Alternating Current Solenoid in Freeze Tests..... 588
- BLACK, G. S. See Webb, G. A.
- BLISS, HARDINO. See Rowe, R. G.
- BLODGETT, GERTRUDE. See Satterlee, H. S.
- BLUE, D. D. See Olsen, A. L.
- BOATNER, C. H., CARAVELLA, MAIZIE, AND KYAME, LILLIAN. Quantitative Determination of Extractable Gossypol in Cottonseed and Cottonseed Meal. Spectrophotometric Method..... 566
- BOERICKE, F. S., AND BANCERT, W. M. Controlled-Atmosphere Induction Melting Furnace for Laboratory..... 302
- BOOGS, W. A. Constant-Level Float Valve..... 201
- Hot Distilled Water Reservoir..... 201
- BOHART, G. S. See Nielsen, J. P.
- BOLIN, D. W., AND STAMBERO, O. E. Rapid Digestion Method for Microdetermination of Phosphorus..... 345
- BOOTH, H. S., AND McNABNEY, RALPH. Improved Fractionating Column for Gases..... 131
- BORUFF, C. S. See Tarnutzer, C. A.
- BOVIE, R. W. See Conner, H. A.
- BOWEN, C. V., AND BARTHEL, W. F. Identification of Nicototine in Tobacco..... 377
- BOYD, T. F. See Weinberg, Sidney.
- BOYD, W. E. Crucible Holder for Use with Rubber Extraction Apparatus..... 721
- Take-Off for Recovering Solvent from Rubber Extraction Apparatus. 722
- BOYER, P. D., SPITZER, ROBERT, JENSEN, CURTIS, AND PHILLIPS, P. H. Determination of Vitamin A and Carotene in Milk. Rapid Extraction Procedure..... 101
- BOYLE, A. J., CASTO, C. C., AND HANEY, R. M. Determination of Magnesia in Magnesite and Dolomite. Potentiometric Method. 313
- HUGHEY, V. V., AND CASTO, C. C. Rapid Estimation of Chlorate Ion Employing Catalysis..... 370
- See also Miller, L. G., and Whitehead, Thomas, Jr.
- BRABSON, J. A., HARVEY, I. W., MAXWELL, G. E., AND SCHAEFFER, O. A. Photometric Determination of Silica in Aluminous Materials by Molybdenum Blue Reaction..... 705
- KARCHMER, J. H., AND KATZ, M. S. Photometric Determination of Phosphorus in Limestone..... 553
- BRADSTREET, R. B. See Lewis, J. B.
- BRADY, L. J. Infrared Analysis of Butadiene..... 422
- BRODE, W. R., PATTERSON, J. W., BROWN, J. B., AND FRANKEL, JEROME. Studies on Chemistry of Fatty Acids. Absorption Spectra Analysis of Conjugation in Fatty Acid..... 77
- BROOKES, M. H. See Hinman, W. F.
- BROWN, F. M. See Tuttle, Clifton.
- BROWN, J. B. See Brode, W. R.
- BROWN, R. E. See Clausen, D. F.
- BROWNE, H. H. Quantitative Method for Determination of Maltose in Presence of Glucose..... 582
- BRYANT, E. F., PALMER, G. H., AND JOSEPH, G. H. Microdetermination of Pectin in Biological Materials. Modification of Pentose-Furfural Method..... 74
- BUNTING, W. E. Determination of Soluble Silica in Very Low Concentrations..... 612
- BURAS, E. M., JR., AND REID, J. D. Simplified Conductometric Titration Apparatus..... 591
- BURCHFIELD, H. P. Identification of Natural and Synthetic Rubbers. 424
- BURNETT, R. S., AND MERRIFIELD, A. L. Device for Renewing Filter-Cake Surface in Small-Scale Vacuum Filtrations..... 365
- BURR, G. O. See Barnes, R. H.
- BURSTEIN, H. N. See Snider, S. R.
- BUSH, M. T., AND GOTH, ANDRES. Laboratory Spray Extraction Column..... 528
- See also Goth, Andres.
- BUSWELL, R. J. See Norris, F. A.
- BUTLER, A. Q. See Wichers, Edward.
- CALDWELL, C. W., PARKER, R. C., AND DIEHL, HARVEY. Apparatus for Automatic Control of Electrodeposition with Graded Cathode Potential..... 532
- CANNON, M. R. Viscosity Measurement. Master Viscometers..... 708
- CANTINO, E. C. Elimination of Nitrate Impurities from 30 Per Cent Hydrogen Peroxide..... 181
- Simple Microtitration Rack..... 346
- CARAVELLA, MAIZIE. See Boatner, C. H.
- CARL, FRED. See Clark, G. L.
- CASE, O. P. Direct Photometric Determination of Silicon in Copper-Base Alloys..... 309
- CASKEY, C. D., JR., AND KNAPP, F. C. Method for Detecting Inadequately Heated Soybean Oil Meal..... 640
- CASSIL, C. C., AND HANSEN, J. W. Colorimetric Analysis of Xanthone Spray Residues..... 35
- CASTO, C. C. See Boyle, A. J.
- CEAOLSKKE, N. H., AND KESSLINGER, S. A. Photoelectric Automatic Liquid Level Control..... 393
- CHAMBERS, W. A., JR. See Singer, Louis.
- CHAPIN, D. S. See Schrenk, W. G.
- CHARKEY, L. W., AND WILGUS, H. S., JR. Chromatographic Determination of Carotene in Alfalfa..... 184
- CHASTAIN, S. M. Device for Rapid Closing of Weighing Bottles..... 579
- CHISHOLM, R. D., AND KOBLITSKY, LOUIS. Modification of Ethanolamine Hydrolysis Method for Determination of Methyl Bromide. 538
- CHOLAK, JACOB, AND HUBBARD, D. M. Microdetermination of Cadmium in Biological Material. Spectrographic, Polarographic, and Colorimetric Methods..... 333
- Spectrochemical Analysis with Air-Acetylene Flame..... 728
- CHU, L. J.-Y. See Lo, Chien-Pen.
- CHUN, H. H. Q. See Sideris, C. P.
- CHURCHILL, J. R. Techniques of Quantitative Spectrographic Analysis..... 653
- CIFONELLI, TONY. Electric Heater for Microprocedures and Melting Points..... 134
- CLARDY, F. B. See McDow, T. B.
- CLARK, G. L., KAYE, W. I., SEABURY, R. L., AND CARL, FRED. Spectrophotometric Study of Oxidation of Quenching Oils..... 740
- CLAUSEN, D. F., AND BROWN, R. E. Determination of Thiamine by Thiochrome Method. Effects of Temperature and Dissolved Oxygen on Fluorescence of Quinine Standard and of Thiochrome..... 572
- CLAYTON, J. O. See Lindeken, C. L.
- CLELAND, J. E., EVANS, J. W., FAUSER, E. E., AND FETZER, W. R. Refractive Index-Dry Substance Tables for Starch Conversion Products..... 161
- COHAN, L. H., SOHN, MADELINE, AND STEINBERG, MARTIN. Determination of Rate of Cure of GR-I and Natural Rubber..... 562
- AND STEINBERG, MARTIN. Determination of Rate of Cure for Natural and Synthetic Rubber..... 15
- COLEMAN, A. M. See Shaw, J. A.
- COLEMAN, G. H. See McCloskey, C. M.
- COLLINS, W. D. See Wichers, Edward.
- COMAR, C. L., MILLER, E. J., RICHARD, M. N., AND BENNE, E. J. Adaptation of Waring Blender for Continuous Emulsification... 717
- CONNER, H. A., AND BOVIE, R. W. Ground Starch as Indicator in Iodometry..... 772
- CONRAD, C. M. Determination of Wax in Cotton Fiber. New Alcohol Extraction Method..... 745
- CONRAD, R. M. See Schrenk, W. G.
- COOK, E. V. See Elliott, J. H.
- CORWIN, A. H. Microchemical Balances. Errors of Kuhlmann Balance..... 258
- COWAN, J. C., FALKENBURG, L. B., AND TEETER, H. M. Analysis of Bodied Drying and Semidrying Oils..... 90
- CRAFT, C. H. See Makepeace, G. R.
- CRAIG, L. C., AND POST, O. W. Improved Apparatus for Solubility Determination or for Small-Scale Recrystallization..... 413
- CROWELL, W. R., AND KÖNIG, OTTO. Improved Microapparatus for Use in Chromatography..... 347
- CURRY, JAMES, AND HUGUS, Z. Z., JR. Circulating Device for Use with Hydrogen Electrode..... 58



DACUS, E. N. Simple Microtorch.....	142	GOODHUE, L. D. See Smith, C. M.	
DASSOW, JOHN. See Stansby, M. E.		GORDON, P. L., AND GILDON, M. A. Determination of Dry Hiding of Pigmented Coatings.....	442
DAVIS, A. R. Physical Methods of Analysis of Synthetic and Natural Rubber (Correction).....	486	GOTH, ANORES, AND BUSH, M. T. Rapid Method for Estimation of Penicillin.....	451
See also Barnes, R. B.		See also Bush, M. T.	
DAVIS, R. O. E. See Yee, J. Y.		GRABOWSKI, H. A. See Straub, F. G.	
DAVISON, J. A. See Lipkin, M. R.		GRAFF, M. M., O'CONNOR, R. T., AND SKAU, E. L. Purification of Solvents for Absorption Spectroscopy. Adsorption Method.....	556
DEAN, E. W., WILLIAMS, A. A., AND FISHER, N. E. Operating Procedure for Determining Heat of Combustion of Gasoline.....	182	GRAVES, STUART. Needed Improvement in Baking Control Methods for Organic Finishes.....	599
DEAN, M. R., AND LEGATSKI, T. W. Specific Gravity of Butadiene...	7	GREEN, L. F. See Zscheile, F. P.	
DEICHMANN, W. B. Phenol Studies. Qualitative Tests for Phenol and <i>o</i> -, <i>m</i> -, and <i>p</i> -Cresol.....	37	GREENBERG, L. A. Bomb Furnace for Carius Digestion.....	308
DEWITT, C. C. See Baten, W. D.		GREENE, L. M. See Beshgetoor, A. W.	
DICKEY, L. W., AND HENRY, ROY. Gum Content of Distillate Diesel Fuels.....	710	GRIFFITH, R. B., AND JEFFREY, R. N. Determining Chlorophyll, Carotene, and Xanthophyll in Plants.....	438
DIEHL, HARVEY. See Caldwell, C. W.		GROSS, S. T., AND MARTIN, D. E. Quantitative Determination of Crystalline Materials by X-Ray Diffraction.....	95
DOTY, P. M., AIKEN, W. H., AND MARK, HERMANN. Water Vapor Permeability of Organic Films.....	686	GUETTEL, C. L. Emission Spectrographic Equipment Used in Quantitative Analysis. Proposed Minimum Requirements.....	670
DOUTLIN, D. R., AND WALLS, W. S. Semiautomatic Pressure Control in Low-Pressure, Low-Temperature Laboratory Fractionation...	40	GUNG, HELEN. See Sears, G. W.	
DOYLE, C. D. Determination of Total Phthalic Anhydride in Modified Alkyd Resins.....	200	GURRY, R. W., AND TRIGG, HASTINGS. Determination of Carbon in Low-Carbon Steel. Precision and Accuracy of Low-Pressure Combustion Method.....	248
DUCKWALL, H. W. See Allen, C. C.		GUTHRIE, J. D. See Hoffpauir, C. L., and Steiner, E. T.	
DUKE, F. R. Color Test for Carbonyl Group.....	110		
Salicylimines as Organic Precipitants. Quantitative Precipitation of Nickel and Copper and Determination of Copper in Brass or Bronze.....	750		
AND STONE, K. G. Preparation of Standard Cerate Solutions from Cerium Titration Residues.....	721	HAAG, H. B. See Larson, P. S.	
DUNBAR, R. E., AND ARNOLO, M. R. Preparation and Reclamation of Copper-Chromium Oxide Catalyst.....	441	HAASIS, F. W. Microstaining Rubber in Ground or Milled Plant Tissues.....	480
DUNPHY, R. A. See Sherry, W. B.		HAINES, G. S. See Williams, Dwight.	
DUSTIN, JAMES. See Mitchell, D. T.		HALL, J. F., JR. See Smaller, Bernard.	
		HALLER, H. L. Some Color Tests for Rotenone Not Specific.....	277
		See also Jacobson, Martin, and Schechter, M. S.	
EARLEY, E. B. Determining Phytin Phosphorus. Stoichiometric Relation of Iron and Phosphorus in Ferric Phytate.....	389	HALLETT, L. T. A.S.T.M. Attracts Large Attendance of Analytical Chemists.....	482
ELLIOTT, J. H., AND COOK, E. V. Determination of Alpha, Para-Dimethylstyrene in Presence of Para-Methylstyrene, Styrene, and Para-Cymene.....	20	HALLIDAY, E. G. See Hinman, W. F.	
ELVING, P. J., AND LAMKIN, J. C. Titrimetric Determination of Zinc. Application to Alloy Analysis.....	194	HAMILTON, P. B., AND ARCHIBALD, R. M. Dialysis Cell for Rapid Quantitative Analytical Microdetermination of Diffusible Components in Blood Plasma.....	136
ENGLIS, D. T., AND HANAHAN, D. J. Determination of Vanillin and Coumarin in Flavoring Extracts. Ultraviolet Absorption Method.....	505	HAMILTON, R. H. Photoelectric Photometry. Analysis of Errors at High and at Low Absorption.....	123
ENGLISH, JAMES, JR. Microapparatus for Purification of Solids....	478	HAMM, W. S. See Shockey, C. F.	
EPPRIGHT, M. A., AND WILLIAMS, R. J. Thiamine Determination. Comparative Study of Yeast-Growth, Yeast-Fermentation, and Thiochrome Methods.....	576	HAMMACK, LOREN, AND NAEGELIN, C. L. Modified Bailey Buret... 357	
ERICKSON, R. H. See Biggs, B. S.		HANAHAN, D. J. See Englis, D. T.	
EVANS, J. W. See Cleland, J. E.		HANEY, R. M. See Boyle, A. J.	
EVANS, R. J., AND ST. JOHN, J. L. Determination of Total Sulfur in Feeds. Modified Nitric and Perchloric Acid Digestion Procedure.....	630	HANSEN, J. W. See Cassil, C. C.	
		HARRISON, R. W. See Stansby, M. E.	
FALKENBURG, L. B. See Cowan, J. C.		HARTIGAN, R. H. See Shaw, J. A.	
FARBER, LIONEL. See Lang, O. W.		HARVEY, E. H. See Petersen, R. B.	
FAUSER, E. E. See Cleland, J. E.		HARVEY, I. W. See Brabson, J. A.	
FEHNEL, E. A., AND AMSTUTZ, E. D. Ethylbis-2,4-dinitrophenylacetate, New pH Indicator. Determination of Saponification Equivalents in Dark-Colored Oils.....	53	HARVEY, W. T. See Lipkin, M. R.	
AND MIRANDA, L. I. Selective Spot Microreaction for Cadmium...	141	HATCH, R. S. Cupriethylene Diamine as Solvent for Precise Determination of Cellulose Viscosity.....	104
FETCHER, E. S., JR. Modifications of Apparatus for Deuterium Oxide Microdetermination by Falling Drop.....	412	HAUGE, S. M. See Zscheile, F. P.	
FETZER, W. R. See Cleland, J. E.		HAWKINGS, R. C., AND THODE, H. G. Polarographic Microdetermination of Copper, Lead, and Cadmium in High-Purity Zinc Alloys.....	71
FILER, L. J., JR. See Mattil, K. F.		HAYWOOD, P. J. C. See Kay, J.	
FISCHER, PHILIP, SPIERS, ROBERT, AND LISAN, PHILIP. Spectrographic Determination of Small Amounts of Tungsten in Steel..	607	HEATH, D. P. See Steffens, Lester.	
FISHER, N. E. See Dean, E. W.		HENNESSY, D. J., WAPNER, SAMUEL, AND TRUHLAR, JOSEPH. Thiamine Content of Pharmaceuticals. Comparative Study of Rat-Curative, Thiochrome, and Fermentation Micromethods.....	476
FLEISHER, HARRY. Sensitive Indicator for Volumetric Determination of Boiler Feedwater Alkalinity (Addition).....	273	HENRY, J. L., AND GELBACH, R. W. Dichromate Determination of Iron, Using Silver Reductor.....	49
FLISIK, H. F. See Swinehart, C. F.		HENRY, R. L. See Zscheile, F. P.	
FORD, LORNE. Support for Flasks on Steam and Water Baths.....	332	HENRY, ROY. See Dickey, L. W.	
FOX, R. P. See Kochakian, C. D.		HILL, R. T., AND JACOBS, W. L. Device to Relieve Bumpy Distillations.....	722
FRANK, R. L. See Ashburn, Gilbert.		HILLSON, H. D. Determination of Large Amounts of Manganese. Modified Persulfate Method.....	560
FRANKEL, JEROME. See Brode, W. R.		HINMAN, W. F., HALLIDAY, E. G., AND BROOKES, M. H. Thiamine in Beef Muscles. Comparison of Values by Thiochrome Reaction Applied with and without Adsorption.....	116
FRANKSTON, J. E. See Albanese, A. A.		HOERR, C. W. See Ralston, A. W.	
FREVEL, L. K. Chemical Analysis by Powder Diffraction.....	209	HOFFPAUIR, C. L., AND GUTHRIE, J. D. Determination of Small Amounts of Sulfate in Cellulose Nitrate and Other Cellulose Esters.....	391
FREY, C. N. See Atkin, Lawrence.		HOIBERG, A. J., AND GARRIS, W. E., JR. Analytical Fractionation of Asphalts.....	294
FRIZZELL, L. D. Quantitative Separations with Exchange Adsorber.	615	HOLLER, A. C., AND YEAGER, J. P. Determination of Sulfur in Brass and Bronze by Combustion Method.....	349
FRONT, JACQUELINE. Support for Kjeldahl Flasks.....	324	HOLMES, F. E. Fractionating Column for Continuous Production of Distilled Water of High Purity.....	748
FRUNDT, R. J. L. See Reed, Gerald.		HOLOWCHAK, JOSEPH. See Rehner, John, Jr.	
FRYLING, C. F. Emulsion Polymerization of Synthetic Rubber in 10-Gram Systems. Experimental Technique.....	1	HONOLD, EDITH, AND WAKEHAM, HELMUT. Analysis Data for Ternary System Acetone-Benzene-Water.....	499
FULMER, E. I., KOLFENBACH, J. J., AND UNDERKOFER, L. A. Quantitative Determination of Mixtures of Methyl Vinyl Ketone and Diacetyl. Dropping Mercury Electrode Method.....	469	HOOD, S. L., PARKS, R. Q., AND HURWITZ, CHARLES. Mineral Contamination Resulting from Grinding Plant Microsamples.....	202
See also Kolfenbach, J. J.		HUBBARD, D. M. See Cholak, Jacob.	
FURBEE, K. D. See McDow, T. B.		HUGHEY, V. V. See Boyle, A. J.	
FURMAN, N. H. See Stone, K. G.		HUGONET, J. J. See Seaman, Wm.	
FURNAS, C. C. See Rowe, R. G.		HUGUS, Z. Z., JR. See Curry, James.	
		HUME, D. N., AND KOLTHOFF, I. M. Iodometric Determination of Iodates, Bromates, or Permanganates in Presence of Copper. Determination of Copper in Presence of Oxidizing Agents.....	103
GARRIS, W. E., JR. See Hoiberg, A. J.		HURWITZ, CHARLES. See Hood, S. L.	
GAWRON, OSCAR, AND BERG, RUTH. Estimation of Vitamin C in Presence of Iron Salts. Stepwise Determination of Vitamin C and Ferrous Iron with Dichlorophenolindophenol.....	757		
GAYLE, F. L. See Schwartz, M. C.		IRBY, VIRGINIA. See Albanese, A. A.	
GEE, E. A. See Olsen, A. L.			
GELBACH, R. W. See Henry, J. L.		JACOBS, M. B. See Goldstone, N. I.	
GENUNG, L. B. See Malm, C. J.		JACOBS, W. L. See Hill, R. T.	
GIBBS, R. S. See Baker, Irvin		JACOBSON, MARTIN, AGREE, FRED, JR., AND HALLER, H. L. Determination of Sesamin.....	166
GIESECKE, PAUL. See Barnes, R. B.		JANSEN, E. F., WAISBROT, S. W., AND RIETZ, EDWARD. Errors in Zeisel Methoxyl Values for Pectin Due to Retained Alcohol.....	523
GILDON, M. A. See Gordon, P. L.		JEFFREY, R. N. See Griffith, R. B.	
GOLDBERG, A. I. Determination of Total Phthalic Anhydride in Oil-Modified Alkyd Resins.....	198	JELINEK, V. C. Flow Characteristics of Dispersions of Cotton and Regenerated Cellulose Rayon Fabrics in Cuprammonium. Their Significance in Fluidity Calculations.....	172
GOLDSTONE, N. I., AND JACOBS, M. B. Chlorometry. Titrimetric Procedure Available for Microanalysis.....	206		
GONICK, HARRY, AND MILANO, J. J. Determination of Tetraethyllead in Gasoline.....	4		



JENSEN, CURTIS. See Boyer, P. D.	
JOHNSON, C. M. See Prater, A. N., and Williams, K. T.	
JOHNSON, M. J. Determination of 2,3-Butylene Glycol in Fermentations. . . . .	626
See also Perlman, David.	
JONES, A. L. See Yoe, J. H.	
JONES, R. G. Purification and Gravimetric Determination of 1-Diethylamino-4-aminopentane. . . . .	431
JONNARD, RAYMOND. Design of Large-Size Laboratory Extraction Glass Apparatus. . . . .	61
JOSEPH, G. H. See Bryant, E. F.	
Joy, A. B. Determination of Potassium in Fertilizer Mixtures. Removal of Ammonia and Organic Matter without Ignition. . . . .	383
KALLMANN, SILVE. Determination of Lithium in Its Minerals. . . . .	712
KAMLET, JONAS. Qualitative Differentiation of Methylcarbinols and Methyl Ketones. . . . .	362
KARCHMER, J. H. See Brabson, J. A.	
KATZ, M. S. See Brabson, J. A.	
KAY, J., HAYWOOD, P. J. C., AND SEAMAN, WM. <i>o</i> -Cresol in Phenol (Correspondence). . . . .	772
KAYE, W. I. See Clark, G. L.	
KEEN, W. N. See Bimmerman, H. G.	
KESSELER, S. A. See Ceaglske, N. H.	
KIESELBACH, RICHARD. Funnel for Use with Standard Taper Flasks. Joining Plastic Tubing to Glass. . . . .	293
Microdetermination of Nitrates by Devarda Method. . . . .	275
Microdetermination of Nitric Oxide in Gases. . . . .	764
Simplified Fritted-Glass Bubbler. . . . .	766
KING, W. H., AND LUHORN, D. A. Determination of Fluoride. Detection and Determination of Acutely Toxic Quantities in Foods and Biological Material. . . . .	538
See also Schrenk, W. G., and Silker, R. E.	457
KIPNIS, FRANK. Separation of Catalysts from Hydrogenation Reaction Mixtures. . . . .	637
KITSON, R. E., WITH MELLON, M. G. Color of Aqueous Potassium Dichromate Solutions. . . . .	42
Colorimetric Determination of Germanium as Molybdivergermanic Acid. . . . .	128
Colorimetric Determination of Phosphorus as Molybdivanadophosphoric Acid. . . . .	379
Further Studies of Molybdenum Blue Reaction. . . . .	466
KLEIBER, MAX. See Smith, F. R.	
KLEIN, BERNARD. Microdetermination of Sulfate. Colorimetric Estimation of Benzidine Sulfate Precipitate. . . . .	536
KNAPP, F. C. See Caskey, C. D., Jr.	
KOBE, K. A. Dropping Funnel. . . . .	641
KOBITSKY, LOUIS. See Chisholm, R. D.	
KOCH, J. M. Analysis of Petroleum Oil-Soluble Sodium Sulfonates by Adsorption. . . . .	25
KOCH, LOUIS, MILLIGAN, R. F., AND ZUCKERMAN, SAMUEL. Identification of Some Important Unsulfonated Azo-2-naphthol Dyes. . . . .	755
KOCHAKIAN, C. D., AND FOX, R. P. Microdetermination of Calcium by Titration of Oxalate with Ammonium Hexanitratocerate. . . . .	762
KÖNIG, OTTO. See Crowell, W. R.	
KOLB, J. J. Stability of Standard Solutions of Copper Perchlorate and Potassium Iodate. . . . .	38
KOLFENBACH, J. J., KOOL, E. R., FULMER, E. I., AND UNDERKOFER, L. A. Laboratory Continuous Countercurrent Liquid-Liquid Extractor. . . . .	473
See also Fulmer, E. I.	
KOLTHOFF, I. M. See Hume, D. N., and Watters, J. I.	
KONEN, H. J. See McQuarrie, E. B.	
KOOL, E. R. See Kolfenbach, J. J.	
KOUTEN, J. W., SHOHAN, J. B., AND MUNN, W. F. Compact Field Apparatus for Determination of Lewisite or Mustard Gas. . . . .	255
KRIEGER, K. A. Apparatus for Surface Area Measurement. . . . .	398
KURTZ, S. S., JR. See Lipkin, M. R.	
KYAME, LILLIAN. See Boatner, C. H.	
LADD, W. A. Electron Microscope Studies of Colloidal Carbon in Vulcanized Rubber. . . . .	642
LAForge, F. B. See Barthel, W. F.	
LAMKIN, J. C. See Elving, P. J.	
LANG, O. W., FARBER, LIONEL, BECK, CLYDE, AND YERMAN, FRED. Determination of Spoilage in Protein Foodstuffs, with Particular Reference to Fish. . . . .	490
LANGDON, W. M., AND O'BRIEN, G. M., JR. General Utility Laboratory Distillation Column. . . . .	639
LANNING, J. H., AND ROSZMANN, C. A. Estimation of Pyridine Content of Pyridine-Acetic Acid Mixture Used in Riboflavin Determination. . . . .	583
LARDY, H. A. See Perlman, David.	
LARSON, I. O. See Miceli, A. S.	
LARSON, P. S., AND HAAG, H. B. Quantitative Determination of Nicotine and Nicotinic Acid in Mixtures. . . . .	86
LEGATSKI, T. W. See Dean, M. R.	
LE ROY, D. J., AND STREACIE, E. W. R. Microdetermination of Nitric Oxide Using Solid Reagents. . . . .	341
LEVINE, JOSEPH. Microchemical Identification of Demerol. . . . .	408
See also Wollner, H. J.	
LEVINE, W. S., AND SEAMAN, HENRY. Electrolytic Determination of Copper in Steel and Cast Iron with Supplementary Colorimetric Procedure for Certain Alloy Steels. . . . .	80
LEWIS, J. B., AND BRADSTREET, R. B. Substitution of Bromine When Determining Unsaturation of Straight and Branched-Chain Olefins. . . . .	617
LIEBER, EUGENE. See Rice, H. T.	
LIEBHAFSKY, H. A. The (Predictable) Concentrating of Standard Solutions Owing to Evaporation. . . . .	349
LINDEKEN, C. L., CLAYTON, J. O., AND SKOGG, D. A. Determination of Ethyl Acetate in Presence of Acetaldehyde. . . . .	734
LINDSAY, W. N., AND MANSFIELD, TOM. Determination of Moisture in Whole Egg Powder. . . . .	628
LINGANE, J. J. Apparatus for Rapid Polarographic Analysis. . . . .	329
Systematic Polarographic Metal Analysis. Analysis of Copper Group with Aid of Electrolytic Separations. . . . .	147
LIPKIN, M. R., DAVISON, J. A., HARVEY, W. T., AND KURTZ, S. S., JR. Pycnometer for Volatile Liquids. Control of Diffusion as Aid in Precision Pycnometry. . . . .	55
LISAN, PHILIP. See Fischer, Philip.	
LITTLE, R. W. Destructive Irradiation Technique of Spectrophotometric Vitamin A Assay. . . . .	288
LITTMAN, H. Z. Fixing Rubber Connections. . . . .	348
LO, CHIEN-PEN, AND CHU, L. J.-Y. Qualitative Study of Color Reaction of Phosphomolybdic Acid. . . . .	637
LONG, W. D. Versatile Liquid-Liquid Extractor. . . . .	180
LOVELL, E. L. Viscometric Chain Length of Wood Cellulose in Triton F Solution. . . . .	683
LUCAS, E. H. Microdetermining Ascorbic Acid in Large Numbers of Plant Samples. . . . .	649
LUCE, E. N. See Marquardt, R. P.	
LUCKMANN, F. H. See Neal, R. H.	
LUHORN, D. A. See King, W. H.	
LUKE, C. L. Determination of Antimony in Tin-Base Alloys. . . . .	448
LYKKEEN, LOUIS, PORTER, PAUL, RULIFFSON, H. D., AND TUEMMLER, F. D. Potentiometric Determination of Acidity in Highly Colored Materials. Application to New and Used Petroleum Lubricants Containing Additives. . . . .	219
McAFEE, JERRY. Positive Displacement Flowmeter. . . . .	303
McARDLE, E. H., AND ROBERTSON, A. E. Evaporation Indices of Hydrocarbon Thinners. . . . .	690
McCLOSKEY, C. M., SUNDBERG, R. L., AND COLEMAN, G. H. Vacuum-Jacketed Ground-Glass Joint for High-Vacuum Distillations at Elevated Temperatures. . . . .	94
McDOW, T. B., FURBEE, K. D., AND CLARDY, F. B. Determination of Tin by Modified Iodometric Method. . . . .	555
McKEE, L. G. See Shockey, C. F.	
McKINLEY, J. B., NICKELS, J. E., AND SIDHU, S. S. Phenyl Isocyanate Derivatives of Certain Alkylated Phenols. Melting Points and X-Ray Powder Diffraction Data. . . . .	304
McKINNEY, D. S., AND AMOROSI, A. M. Determination of Carbon Dioxide in Water. . . . .	315
MACKINNEY, G. See Prater, A. N.	
McLENDON, VERDA. See Olsen, A. L.	
McMILLAN, W. R. Improved Timing Siphon. . . . .	592
McNABBE, W. M. See Snyder, M. D.	
McNABNEY, RALPH. See Booth, H. S.	
McQUARRIE, E. B., AND KONEN, H. J. Glass Electrode Assembly for Titrating Microbiological Vitamin Assays. . . . .	205
MAGES, STEPHEN. See Wachs, Herman.	
MAGNUSON, H. J., AND WATSON, E. B. Microdetermination of Arsenic in Biological Materials. . . . .	339
MAKEPEACE, G. R., AND CRAFT, C. H. Colorimetric Determination of Nickel in Steel. . . . .	375
MALM, C. J., GENUNG, L. E., WILLIAMS, R. F., JR., AND PILE, M. A. Analysis of Cellulose Derivatives. Total Acyl in Cellulose Organic Esters by Saponification in Solution. . . . .	501
MANSFIELD, TOM. See Lindsay, W. N.	
MARK, HERMANN. See Doty, P. M.	
MARKHAM, A. E. Determination of Noncondensables in Gas. . . . .	681
MARQUARDT, R. P., AND LUCE, E. N. Determination of <i>o</i> -Xylene in Recycle Styrene. . . . .	751
MARTIN, D. E. See Gross, S. T.	
MASSAD, E. A. See Seaman, Wm.	
MATCHETT, J. R. See Wollner, H. J.	
MATTIL, K. F., AND FILER, L. J., JR. Determination of Gallic Acid Added to Fats and Oils. . . . .	427
MAXWELL, G. E. See Brabson, J. A.	
MELLON, M. G. See Kitson, R. E.	
MENDENHALL, E. E. See Wilson, J. L.	
MERRIFIELD, A. L. See Burnett, R. S.	
MERRITT, L. L., JR. Determination of Small Amounts of Zinc by Measurement of Fluorescent Turbidities. . . . .	758
AND WALKER, J. K. 8-Hydroxyquinoline as Analytical Reagent. . . . .	387
MESSINGER, P. H. See Wichers, Edward.	
METZLER, D. E., MYERS, R. J., AND SWIFT, E. H. Use of Iodine Monochloride in Standardization of Permanganate Solutions with Arsenious Oxide. . . . .	625
MICELI, A. S., AND LARSON, I. O. Determination of Zinc in Cyanide Brass-Plating Baths. . . . .	665
MILANO, J. J. See Gonick, Harry.	
MILLER, E. J. See Comar, C. L.	
MILLER, E. S. See Barnes, R. H.	
MILLER, L. G., BOYLE, A. J., AND NEILL, R. B. Routine Determination of Zinc in Magnesium Alloys. Volumetric Method. . . . .	256
MILLER, MARTIN. See Baker, Irvin.	
MILLIGAN, R. F. See Koch, Louis.	
MIRANDA, L. I. See Feigl, Fritz.	
MITCHELL, D. T., SHILDNECK, PAUL, AND DUSTIN, JAMES. Laboratory-Size Glass Circulating Evaporator. . . . .	754
MITCHELL, H. H. Determination of Nutritive Value of Proteins of Food Products. . . . .	696
MITCHELL, JOHN, JR., SMITH, D. M., AND MONEY, F. S. Semimicrosaponification of Esters. . . . .	410
MONEY, F. S. See Mitchell, John, Jr.	
MORRIELLO, CHARLES. See Wachs, Herman.	
MORRIS, D. L. Observation of Possible Value for Sugar Determinations. (Correction, 693). . . . .	537
MOSHIER, R. W. See Whitnack, G. C.	
MUNN, W. F. See Kouten, J. W.	
MURRAY, W. M., JR., AND ASHLEY, S. E. Q. Determination of Carbon by Low-Pressure Combustion Method. . . . .	242
AND NIEDRACH, L. W. Determination of Carbon. Simplification of Low-Pressure Combustion Apparatus. . . . .	634
MYERS, R. J. See Metzler, D. E.	
NAEGELIN, C. L. See Hamnack, Loren.	
NASH, H. A. See Zscheile, F. P.	
NEAL, R. H., AND LUCKMANN, F. H. Determination of Vitamin A Content of Margarine. Spectrophotometric Method. . . . .	358
NEILL, R. B. See Miller, L. G.	
NICHOLS, M. L., AND ROOERS, L. H. Microdetermination of Small Amounts of Molybdenum in Plants and Soils. . . . .	137
NICKELS, J. E. See McKinley, J. B.	
NIEDRACH, L. W. See Murray, W. M., Jr.	
NIELSEN, J. P., AND BOHART, G. S. Determination of Crude Lipid in Vegetable Matter. . . . .	701



NORRIS, F. A., AND BUSWELL, R. J. Stability of Wijs Solution for Iodine Number Determinations.....	417	SCHWARTZ, M. C., AND GAYLE, F. L. Constant-Level Feeder for Continuous Evaporation in Determination of Total Solids.....	120
NORTON, A. R. See Seaman, Wm.		SEABURY, R. L. See Clark, G. L.	
NORWICK, BRAHAM. Textile Finishes and Fiber Identification Stains.....	274	SEAMAN, HENRY. Colorimetric Determination of Nickel in Bronze..	354
		See also Levine, W. S.	
O'BRIEN, G. M., JR. See Langdon, W. M.		SEAMAN, WM., NORTON, A. R., WOODS, J. T., AND HUGONET, J. J. Spectrophotometric Microdetermination of Leuco Crystal Violet after Oxidation with Benzoyl Peroxide.....	336
O'CONNOR, R. T. See Graff, M. M.		NORTON, A. R., WOODS, J. T., AND MASSAD, E. A. Analysis of Acetylsulfanilyl Chloride by Karl Fischer Reagent.....	517
OGBURN, S. C. See Sherry, W. B.		See also Kay, J.	
OLSEN, A. L., GEE, E. A., AND MCLENDON, VERDA. Precision and Accuracy of Colorimetric Procedures as Analytical Control Methods. Determination of Aluminum.....	169	SEARS, G. W., AND GUNG, HELEN. Use of Color Indicator in Tannin Method for Determination of Beryllium and Aluminum.....	598
GEE, E. A., MCLENDON, VERDA, AND BLUE, D. D. Precision and Accuracy of Colorimetric Procedures as Analytical Control Methods. Determination of Silica.....	462	SHAEFER, W. E. Analysis of Solutions of Ethyl Ether, Benzene, Ethyl Alcohol, and Water.....	432
OSBORN, R. A. See Wichers, Edward.		SHANTZ, E. M. Antimony Trichloride Reaction of Vitamin D.....	179
PAGEL, H. A. Carbon Dioxide Generator for Dumas Micro-method of Determining Nitrogen.....	344	SHAW, J. A., HARTIGAN, R. H., AND COLEMAN, A. M. Determination of Hydrocyanic Acid, Especially in Coke-Oven Gas.....	550
PALMER, G. H. See Bryant, E. F.		SHAW, T. P. G. Systematic Procedure for Identification of Synthetic Resins and Plastics.....	541
PARKER, R. C. See Caldwell, C. W.		SHERRY, W. B., SWINEHART, C. F., DUNPHY, R. A., AND OGBURN, S. C. Methods of Analysis for Anhydrous Hydrofluoric Acid. Manufacturing Chemists' Association Committee Report.....	483
PARKS, R. Q. See Hood, S. L.		SHILNECK, PAUL. See Mitchell, D. T.	
PATTERSON, G. D., AND SLOAN, C. K. Laboratory Evaluation of Corrosion-Inhibitive Pigments.....	234	SHOCKEY, C. F., MCKEE, L. G., AND HAMM, W. S. Instrument for Measuring Changes in Texture of Dehydrated Fish.....	638
PATTERSON, J. W. See Brode, W. R.		SHOHAN, J. B. See Kouten, J. W.	
PEARL, I. A. Continuous Liquid-Liquid Extractor.....	62	SHREWSBURY, C. L. See Zscheile, F. P.	
PEARLSON, W. H. Vacuum Stopcock Lubricant Unaffected by Hydrocarbons.....	415	SHUMAN, A. C. Apparatus for Measuring Gas Permeability of Film Materials of Low Permeability.....	58
PERLMAN, DAVID, LARDY, H. A., AND JOHNSON, M. J. Determination of Citric Acid in Fermentation Media and Biological Materials.....	515	SIDERIS, C. P., YOUNG, H. Y., AND CHUN, H. H. Q. Improvements in Determination of Iron by Nitroso R Salt Method.....	276
PETERSEN, G. W., AND RADKE, H. H. Microdetermination of Small Amounts of Acrylonitrile in Air.....	63	SIDHU, S. S. See McKinley, J. B.	
PETERSEN, R. B., AND HARVEY, E. H. Colorimetric Method for Determination of Ergosterol.....	495	SILKER, R. E., SCHRENK, W. G., AND KING, H. H. Determination of Carotene in Dehydrated Alfalfa. Simplified Method.....	513
PHILLIPS, P. H. See Boyer, P. D.		See also Schrenk, W. G.	
PILE, M. A. See Malm, C. J.		SILVERMAN, SHIRLEIGH. Photoelectric Photometer for Determining Carbon Disulfide in Atmosphere (Correction).....	250
PLATNER, W. S. Stable Starch Solution for Dissolved Oxygen Determinations.....	369	SINGER, LOUIS, AND CHAMBERS, W. A., JR. Colorimetric Determination of Chromium in Steel.....	507
PLEIN, E. M., AND POE, C. F. Determination of Camphor and Alcohol in Spirit of Camphor by Refractive Index and Specific Gravity.....	168	SKAU, E. L. See Graff, M. M.	
POE, C. F. See preceding item		SKOOG, D. A. See Lindeken, C. L.	
POOL, M. F. See Prater, A. N.		SLOAN, C. K. See Patterson, G. D.	
PORTER, PAUL. See Lykken, Louis.		SMALLER, BERNARD, AND HALL, J. F., JR. Spectrophotometric Microdetermination of Iodine Liberated in Oxidation of Carbon Monoxide by Iodine Pentoxide.....	64
POST, O. W. See Craig, L. C.		SMITH, C. M., AND GOODHUE, L. D. Increase in Concentration of Insecticide in Freon-12 Accompanying Transfer or Discharge of Aerosol-Producing Solution.....	355
PRATER, A. N., JOHNSON, C. M., POOL, M. F., AND MACKINNEY, G. Determination of Sulfur Dioxide in Dehydrated Foods.....	153	SMITH, D. M. See Mitchell, John, Jr.	
PRUTTON, A. J. See Barton, C. J.		SMITH, F. R., AND KLEIBER, MAX. Apparatus for Measuring Rate of Gas Penetration through Food-Packaging Materials.....	586
PUDDINGTON, I. E. Simple Automatic Pump for Collecting Gases at Low Pressures.....	592	SMITH, G. F., AND RICHTER, F. P. Substituted 1,10-Phenanthroline Ferrous Complex Oxidation-Reduction Indicators. Potential Determinations as Function of Acid Concentration.....	580
Stopcock Lubricants for Use with Organic Vapors.....	415	SMITH, S. B., AND STREMPFER, J. F. Determination of Phthalate.....	416
RADER, G. A. Safety Cap for Laboratory Glass Distillation Equipment.....	276	SNIDER, S. R., AND BURSTEIN, H. N. Detection of Destructively Distilled Wood Turpentine in Other Kinds of Turpentine by Means of Aniline Point.....	603
RADKE, H. H. See Petersen, G. W.		SNYDER, J. C., AND STEUBER, WALTER. Simple Constant Reflux Take-Off for Distillation Systems.....	454
RALSTON, A. W., AND HOERR, C. W. Quantitative Determination of High Molecular Weight Primary Aliphatic Amines.....	459	SNYDER, M. D., AND McNABB, W. M. Semimicrodetermination of Arsenic in Insecticides.....	414
REED, GERALD, WISE, E. C., AND FRUNDT, R. J. L. Quantitative Separation of Alcohol and Ester Forms of Vitamin A by Solvent Extraction and Chromatographic Methods.....	509	SOHN, MADELINE. See Cohan, L. H.	
REHNER, JOHN, JR., AND HOLOWCHAK, JOSEPH. Determination of Total and Combined Sulfur in Butyl Ruhher.....	98	SOLOMON, ERNEST. Analysis of n-Butane-Isobutane Mixtures by Density Method.....	348
REID, J. D. See Buras, E. M., Jr.		SPATT, CARL, AND SCHNEIDER, FRANK. Microtechnique of Qualitative Organic Analysis. Identification of Organic Acids by Partition Method.....	479
RICE, H. T., AND LIEBER, EUGENE. Furfural Solution Temperatures of Hydrocarbons. Evaluation of Mixed Aniline Point Determination and Application of Furfural.....	107	SPECK, R. M. See Turer, Jack.	
RICHARD, M. N. See Comar, C. L.		SPIERS, ROBERT. See Fischer, Philip.	
RICHTER, F. P. See Smith, G. F.		SPITZER, ROBERT. See Boyer, P. D.	
RIEMAN, WM., III. New Type of Microburet.....	475	STAMBERG, O. E. See Bolin, D. W.	
RIETZ, EDWARD. See Jansen, E. F.		STANDEN, G. W. Qualitative Spectrographic Analysis.....	675
RITTSCHOF, L. A. See Tarnutzer, C. A.		STANSBY, M. E., HARRISON, R. W., DASSOW, JOHN, AND SATER, MARIE. Determining Volatile Bases in Fish. Comparison of Precision of Certain Micromethods.....	593
ROBERTSON, A. E. See McArdle, E. H.		STEACIE, E. W. R. See Le Roy, D. J.	
ROBINSON, F. B. Pigment Determination in Carbon Black and Iron Blue Paints.....	364	STEFFENS, LESTER, AND HEATH, D. P. Automatic Distillation Apparatus for Gasoline Analysis.....	525
ROGERS, H. R. Furfural Determination. Iodine Method for Hydrolyzed Wood Liquors.....	319	STEINBERG, MARTIN. See Cohan, L. H.	
ROGERS, L. H. See Nichols, M. L.		STEINER, E. T., AND GUTHRIE, J. D. Determination of Starch in Sweet Potato Products and Other Plant Materials.....	736
ROSIN, JOSEPH. See Wichers, Edward.		STENGER, V. A. See Beshgetoor, A. W.	
ROSS, J. F. See Wichers, Edward.		STEUBER, WALTER. See Snyder, J. C.	
ROZSMANN, C. A. See Lanning, J. H.		STONE, K. G., AND FURMAN, N. H. Polarographic Use of Micro-organic Reagents. Magnesium with 8-Hydroxyquinoline.....	596
ROWE, R. G., FURNAS, C. C., AND BLISS, HARDING. Iodine Number Method for Tall Oil.....	371	See also Duke, F. R.	
RUDERMAN, I. W. Versatile Continuous Laboratory Extractor....	332	STRAUB, F. G., AND GRABOWSKI, H. A. Photometric Determination of Silica in Condensed Steam in Presence of Phosphates.....	574
RULIFFSON, H. D. See Lykken, Louis.		STREMPFER, J. F. See Smith, S. B.	
RUSOFF, I. I. See Barnes, R. H.		STUBBLEFIELD, F. M. Determination of Nitrogen Dioxide by Cerate Oxidimetry.....	366
ST. JOHN, J. L. See Evans, R. J.		SUEN, TZENG-JIUEQ, AND WANG, MAO-CHIEN. Estimation of Tung Oil as Adulterant.....	511
SANDELL, E. B. Colorimetric Determination of Traces of Osmium.....	342	SUNDBERG, R. L. See McCloskey, C. M.	
SATER, MARIE. See Stansby, M. E.		SWAIN, L. A. Carr-Price Reagent Dispenser.....	241
SATTERLEE, H. S., AND BLODGETT, GERTRUDE. Ultramicrodetermination of Arsenic by Gutzeit Spot-Filtration under Vacuum. Rapid Technique Employing Photometric Calibration and Permanent Photographic Standards.....	400	SWEENEY, W. J. Applicability of Newer Physical Methods for Hydrocarbon Analysis.....	723
SCHAEFFER, O. A. See Brahsen, J. A.		SWERDLOW, M. A. See Vestling, C. S.	
SCHAIBLE, P. J. See Bandemer, S. L.		SWIFT, E. H. See Metzler, D. E.	
SCHUCHTER, M. S., AND HALLER, H. L. Colorimetric Determination of 1-Chloro-2,4-dinitrobenzene as Impurity in 2,4-Dinitroanisole. Colorimetric Determination of 2,4-Dinitroanisole.....	326	SWINEHART, C. F., AND FLISIK, H. F. Sampling and Analysis of Anhydrous Hydrogen Fluoride.....	419
Weighing Funnels.....	133	See also Sherry, W. B.	
SCHLEY, C. R. Apparatus for Washing Selas Crucibles.....	539	SYCHEFF, V. M. Extraction of Oil and Vitamin A in Shark Liver Analysis. Xylene-Centrifuge Method.....	126
Facilitating Reading of Volumes in Determinations of Moisture by Distillation.....	720		
SCHNEIDER, FRANK. See Spatt, Carl.		TARNUTZER, C. A., RITTSCHOF, L. A., AND BORUFF, C. S. Absorption Spectra of Volatile Essential Oils. Detection of Alpha-Dicarbonyl Compounds.....	621
SCHRENK, W. G., CHAPIN, D. S., AND CONRAD, R. M. Determination of Vitamin A in Dehydrated Eggs.....	632	TEETER, H. M. See Cowan, J. C.	
SILKER, R. E., AND KING, H. H. Effects of Beta-Carotene Isomerization on Its Absorption at 326 Millimicrons.....	328	THACHER, H. C., JR. Nomographic Chart for Correcting Weights to Vacuum.....	275
See also Silker, R. E.			
SCHULTZ, A. S. See Atkin, Lawrence.			



THODE, H. G. See Hawkings, R. C.	
THOMPSON, J. B. Microdetermination of Iron in Food Products....	646
THORNTON, W. M., JR. Precise Measurement of Volume in Titrimetric Analysis.....	50
TODD, FLOYD. New Design of Humidity Cabinet for Corrosion Testing.....	394
TOKOS, J. V. See West, P. W.	
TOWNE, R. S. Precision Head for Small Fractionating Columns....	584
TRIEBOLD, H. O. See Althouse, P. M.	
TRIGG, HASTINGS. See Gurry, R. W.	
TRUHLAR, JOSEPH. See Hennessy, D. J.	
TUEMMLER, F. D. See Lykken, Louis.	
TURER, JACK, AND SPECK, R. M. Determination of Fatty Acid Monoesters of <i>l</i> -Ascorbic and <i>d</i> -Isoascorbic Acids in Fats and Oils.	464
TUTTLE, CLIFTON, AND BROWN, F. M. Device for Projecting Microimage of Reading Scale.....	645
UNDERKOFER, L. A. See Fulmer, E. I., and Kolfenbach, J. J.	
VESTLING, C. S., AND SWERDLOW, M. A. Use of Synthetic Detergents in Van Slyke Determination of Oxygen Capacity.....	581
WACHS, HERMAN, MORRIELLO, CHARLES, AND MAGES, STEPHEN. Determination of Freon-Insoluble Solids in Twenty Per Cent Pyrethrum Extracts.....	453
WADDLE, H. M. Improved Distillation Receiver.....	537
WAGNER, D. L. See Albanese, A. A.	
WAGNER, R. H. Osmometry of High-Polymer Solutions. Apparatus.	520
WAISBROT, S. W. See Jansen, E. F.	
WAKEHAM, HELMUT. See Honold, Edith.	
WALKER, A. O. Funnel for Filling Microcapillaries.....	343
WALKER, J. K. See Merritt, L. L., Jr.	
WALLS, W. S. See Douslin, D. R.	
WANG, MAO-CHIEN. See Suen, Tzeng-Jueq.	
WAPNER, SAMUEL. See Hennessy, D. J.	
WATSON, E. B. See Magnuson, H. J.	
WATTERS, J. I., AND KOLTHOFF, I. M. Determination of Manganese after Oxidation to Tri-Dihydrogen Pyrophosphatomanganate. Use of Pyridine to Separate Iron, Chromium, Vanadium, and Cerium from Manganese.....	187
WEATHERBURN, A. S., WEATHERBURN, M. W., AND BAYLEY, C. H. Determination of Copper and Zinc in Their Naphthenates.....	703
WEATHERBURN, M. W. See preceding item.	
WEBB, G. A., AND BLACK, G. S. Determining Hydrogen in Gases with Thermal-Conductivity Apparatus.....	719
WEINBERG, SIDNEY, AND BOYD, T. F. Rapid Determination of Zinc in Magnesium Alloys.....	460
WESSLER, ALFRED. Determination of Germanium in Steel.....	311
WEST, P. W., AND TOKOS, J. V. Detection of Bismuth by Means of Bruce Citrate.....	761
WHITE, C. E. Benzoin as Fluorescent Reagent for Zinc (Correction).	102
WHITE, E. P. Thiosulfate Washers in Alkoxy Microdeterminations.	207
WHITE, J. W., JR. See Zscheile, F. P.	
WHITEHEAD, THOMAS, JR., AND BOYLE, A. J. Spectrographic Method for Small Amounts of Calcium in Magnesium Metal.....	455
WHITNACK, G. C., AND MOSHIER, R. W. Determination of Formaldehyde in Presence of Acrolein and Other Aldehydes by Polarographic Method. (Correction, 693).....	496
WICHERS, EDWARD, BUTLER, A. Q., COLLINS, W. D., MESSINGER, P.	
H., OSBORN, R. A., ROSIN, JOSEPH, AND ROSS, J. F. Recommended Specifications for Analytical Reagent Chemicals. Acid Hydriodic with Preservative, Acid Perchloric, Dimethylglyoxime, Lead Subacetate (for Sugar Analysis), Manganese Sulfate Monohydrate, Mercuric Oxide Yellow, Phosphorus Pentoxide, Sodium Phosphate Dibasic Heptahydrate, Zinc.....	281
WILGUS, H. S., JR. See Charkey, L. W.	
WILLARD, H. H., AND ZUEHLKE, C. W. Gravimetric and Volumetric Determination of Germanium.....	322
WILLIAMS, A. A. See Dean, E. W.	
WILLIAMS, DWIGHT, AND HAINES, G. S. Alundum Gas Diffusers....	680
Determination of Sodium in Potassium Hydroxide.....	157
WILLIAMS, K. T., AND JOHNSON, C. M. Determination of Soluble Pectin and Pectic Acid by Electrodeposition.....	23
WILLIAMS, R. F., JR. See Malm, C. J.	
WILLIAMS, R. J. See Eppright, M. A.	
WILLIAMS, VAN ZANDT. See Barnes, R. B.	
WILLIAMS, W. L. See Atkin, Lawrence.	
WILSON, J. L., AND MENDENHALL, E. E. Measurement of Detergency: Determination of Rate of Hard Water Film Formation in Washing of Glass Objects, 253; Photometer for Determination of Films on Transparent Surfaces.....	251
WISE, E. C. See Reed, Gerald.	
WISE, L. E., AND APPLING, J. W. Quantitative Determination of <i>d</i> -Galactose by Selective Fermentation with Special Reference to Plant Mucilages.....	28
WOLF, BENJAMIN. Determination of Nitrate, Nitrite, and Ammonium Nitrogen. Rapid Photometric Determination in Soil and Plant Extracts.....	446
Rapid Photometric Determination of Total Nitrogen, Phosphorus, and Potassium in Plant Material.....	121
WOLLNER, H. J., MATCHETT, J. R., AND LEVINE, JOSEPH. Fractionating Molecular Still.....	529
WOODS, J. T. See Seaman, Wm.	
YEAGER, J. P. See Holler, A. C.	
YEE, J. Y. Determining Hygroscopicity of Fertilizers.....	367
AND DAVIS, R. O. E. Accelerated Method for Determining Moisture Absorption.....	487
YERMAN, FRED. See Lang, O. W.	
YOE, J. H., AND JONES, A. L. Colorimetric Determination of Iron with Disodium-1,2-dihydroxybenzene-3,5-disulfonate.....	111
Gravimetric Determination of Tungsten with Anti-1,5-di-( <i>p</i> -methoxyphenyl)-1-hydroxylamino-3-oximino-4-pentene.....	45
YOUNG, H. Y. See Sideris, C. P.	
YOUNG, R. S. Monel Metal Pouring Plate for Silica Fusions.....	590
ZENTNER, E. T. Delivery of Liquids at Low and Constant Rates.	471
ZSCHEILE, F. P., AND HENRY, R. L. Spectroscopic Study of Fish Liver Oils in Relation to Vitamin A.....	436
HENRY, R. L., WHITE, J. W., JR., NASH, H. A., SHREWSBURY, C. L., AND HAUGE, S. M. Determination of Vitamin A and Carotenoids in Butterfat. Spectroscopic Characteristics of Butterfat Fractions and Problems Involved in Biological Interpretations.....	190
NASH, H. A., HENRY, R. L., AND GREEN, L. F. Determination of Vitamin A and Carotenoids in Butterfat. Comparison of Direct Spectrophotometry with Filter Photometry and Use of Antimony Trichloride Reaction.....	83
ZUCKERMAN, SAMUEL. See Koch, Louis.	
ZUEHLKE, C. W. See Willard, H. H.	

# SUBJECT INDEX

VOLUME 16—1944

ACETALDEHYDE, Ethyl Acetate Determination in Presence of....	734
Acetic Acid, Pyridine Estimation in Mixture with, for Riboflavin Determination.....	583
Acetone-Benzene-Water System, Analysis Data for.....	499
Acetylene-Air Flame for Spectrochemical Analysis.....	728
Acetylsulfanil Chloride Analysis by Karl Fischer Reagent.....	517
ACIDS	
Determination in Highly Colored Materials. Petroleum Lubricants Containing Additives.....	219
Fatty, Absorption Spectra Analysis of Conjugation in.....	77
Organic, Microidentification by Partition Method.....	479
(See also individual acid.)	
Acrolein, Formaldehyde Determination in Presence of. (Correction, 693).....	496
Acrylonitrile Microdetermination in Air.....	63
Acyl Determination in Cellulose Organic Esters.....	501
Aerosol-Producing Solution. Insecticide Concentration Increased in Freon-12.....	355
AIR	
Acrylonitrile Microdetermination in.....	63
Carbon Disulfide Determination in (Correction).....	250
Carbon Monoxide Determination in, by Iodine Pentoxide Method. Microdetermination of Iodine Liberated.....	64
Contaminant Collection with Midget Impinger.....	346
Alcohol Dehydrogenation Catalysts. Copper-Chromium Oxide Preparation and Reclamation.....	441
(See also Ethanol.)	
Aldehydes. Formaldehyde Determination in Presence of Other Aldehydes. (Correction, 693).....	496
Alfalfa, Carotene Determination in.....	513
Alkalinity of Boiler Water, Indicator for Determination of (Correspondence).....	273
Alkaloids. Demerol Microidentification.....	408
Alkoxy Microdeterminations, Thiosulfate Washers in.....	207
Alkyd Resins, Phthalic Anhydride Determination in.....	198, 200
ALLOYS	
of Aluminum, Quenching Oils for. Spectrophotometric Study of Oil Oxidation.....	740
Brass.....	165, 349, 750
Bronze.....	269, 309, 349, 354, 750
of Copper, Silicon Determination in.....	309
of Devarda, for Nitrate Microdetermination.....	764
of Magnesium, Zinc Determination in.....	256, 460
Monel Metal Pouring Plate for Silica Fusions.....	590
Raney Nickel Separation from Hydrogenation Reaction Mixtures..	637
of Tin, Antimony Determination in.....	448
Tin Determination in, by Iodometric Method.....	555
Zinc Determination in, by Titrimetric Method.....	194
of Zinc, Microdetermination of Copper, Lead, and Cadmium in....	71
(See also Steel.)	
Alumina, Calcined, Silica Determination in, by Molybdenum Blue Reaction.....	705
ALUMINUM	
Alloy-Casting Quenching Oils, Spectrophotometric Study of Oxidation of.....	740
Determination by Colorimetric Methods.....	169, 598
Determination with 8-Hydroxyquinoline.....	387
Silicon Determination in, by Molybdenum Blue Reaction.....	705
Spectrographic Quantitative Techniques for.....	653
Alundum Gas Diffusers.....	680
Amines, Primary Aliphatic, of High Molecular Weight Determined Quantitatively.....	459
Amino Acid Analysis of Vegetables.....	609
4-Aminopentane, 1-Diethylamino-, Purification and Determination..	431
Ammonium Hexanitratocerate for Calcium Microdetermination as Oxalate.....	762
Ammonium Nitrogen Determination in Soil and Plant Extracts by Photometric Method.....	446
Ammonium Quaternary Salts, Colorimetric Assay of.....	739
Aniline Point, Turpentine Adulteration Detected by.....	603
Anisole, 2,4-Dinitro-, Determination of.....	325, 326



Anti-1,5-di-( <i>p</i> -methoxyphenyl)-1-hydroxylamino-3-oximino-4-pentene for Tungsten Determination.....	45	in Milk.....	10
Antimony Determination in Tin-Base Alloys.....	448	in Plants.....	43
ANTIMONY TRICHLORIDE		Carotenoid Determination in Butterfat.....	83, 190
Dispenser for Chloroform Solution of.....	241	Carrots, Dehydrated, Sulfur Dioxide Determination in.....	15
for Gossypol Determination in Cottonseed and Meal.....	566	CATALYSTS	
for Vitamin A and Carotenoid Determination in Butterfat.....	83	for Chlorate Ion Estimation.....	370
for Vitamin D Estimation.....	179	Copper-Chromium Oxide Preparation and Reclamation.....	44
ARSENIC		Hydrogen Determination in Exhaust Gases as Evaluation of Activity of.....	71
Microdetermination in Biological Materials.....	339	Separation from Hydrogenation Reaction Mixtures.....	63
Semimicrodetermination in Insecticides.....	414	Cathode Potential. See Electrodeposition.....	44
Ultramicrodetermination by Gutzeit Spot Filtration under Vacuum.....	400	Celite as Carrier for Copper-Chromium Oxide Catalyst.....	32
Arsenious Oxide in Permanganate Solution Standardization.....	625	Cell for Polarographic Analysis.....	42
<i>l</i> -Ascorbic Acid Esters Determined in Fats and Oils.....	464	CELLULOSE	
Ascorbic Acid Microdetermination in Plant Samples.....	649	Hemi-, Photometric Determination of.....	42
Ashing Procedure.....	417	Molecular Weight. Measurement of Average Degree of Polymerization.....	35
Asphalt, Acidity Determination in, by Potentiometric Method.....	219	Regenerated Rayon Fabric Dispersions in Cuprammonium, Flow Characteristics of.....	17
Asphalt Fractionation by Solvent Separation.....	294	Viscosity Determination with Cupriethylene Diamine as Solvent.....	10
Azo-2-naphthol Dyes, Unsulfonated, Identification of.....	755	from Wood. Viscometric Chain Length in Triton F.....	68
BAILEY Buret for Weighing.....	357	CELLULOSE ESTERS	
Baking Instrument for Organic Finishes, with Automatic Control.....	599	Acyl Determination in, by Saponification in Solution.....	50
Balances, Micro-. Device for Projecting Image of Reading Scale.....	645	Plasticizer Determination in.....	9
Balances, Micro-, of Kuhlmann, Errors in.....	258	Sulfate Determination in.....	39
Base Microdetermination in Fish.....	593	Centrifuge. Adaptor for Angle Centrifuge Tests.....	19
Baths. Flask Support on Steam and Water Baths.....	332	Cerate Oxidimetry for Nitrogen Dioxide Determination.....	36
Beef Muscles, Thiamine Determination in.....	116	Cerate Standard Solutions Prepared from Cerium Titration Residues.....	72
Benzene-Acetone-Water System, Analysis Data for.....	499	Cerium Titration Residues. See preceding item.....	28
Benzene-Ethyl Ether-Ethanol-Water Solutions Analyzed.....	432	Chemicals, Analytical Reagent, Recommended Specifications for.....	37
Benzidine Sulfate Precipitation for Microdetermination of Sulfate.....	536	Chlorate Estimation by Catalysis.....	32
Benzoin as Fluorescent Microreagent for Zinc (Correction).....	102	1-Chloro-2,4-dinitrobenzene Determination as Impurity in 2,4-Dinitroanisole.....	24
Benzoyl Peroxide in Microdetermination of Leuco Crystal Violet.....	336	Chloroform Solution of Antimony Chloride, Dispenser for.....	20
Beryllium Determination by Tannin Colorimetric Method.....	598	Chlorometry as Titrimetric Microprocedure.....	43
BIOLOGICAL MATERIAL		Chlorophyll Determination in Plants.....	34
Arsenic Microdetermination in.....	339	Chromatography, Microapparatus Improved for.....	50
Cadmium Microdetermination in.....	333	Chromium Determination in Steel by Colorimetric Method.....	41
Citric Acid Determination in.....	515	Chromium Oxide-Copper Catalyst Preparation and Reclamation.....	58
Extraction Spray Column for.....	528	Circulating Device for Use with Hydrogen Electrode.....	51
Fluoride Determination in Toxic Quantities.....	457	Citric Acid Determination in Fermentation Media and Biological Materials.....	51
Pectin Microdetermination in.....	74	Coal Proximate Analyses by Discriminant Function.....	3
Spectrochemical Analysis with Air-Acetylene Flame.....	728	Coke-Oven Gas, Hydrocyanic Acid Determination in.....	55
(See also individual biological material.)		COLORIMETRY	
Bismuth Microdetection by Brucine Citrate.....	761	Molybdenum Blue Reaction Studied.....	46
Bismuth. Polarographic Analysis of Copper Group.....	147	Phosphomolybdic Acid Reaction Studied Qualitatively.....	63
Blender of Waring Adapted to Continuous Emulsification.....	717	Potassium Dichromate Solutions as Standards in.....	42
Blood Oxygen-Capacity Determination by Van Slyke Method Using Detergents.....	581	Precision and Accuracy as Control Method: Aluminium Determination, 169; Silica Determination.....	46
Blood Plasma, Dialysis Cell for Microdetermination of Diffusible Components.....	136	COLUMNS	
Boiler Scale, Powder Diffraction of.....	209	Distillation, of General Utility.....	63
Boiler Water, Alkalinity Indicator for (Correspondence).....	273	for Distilled Water of High Purity.....	74
Bomb Furnace for Carius Digestion.....	308	for Extraction by Spray Device.....	52
Book Reviews.....	145, 279, 350, 539, 652, 722	Fractionating, for Gases.....	13
BRASS		Fractionating, Precision Head for.....	58
Copper Determination in, with Salicylimines.....	750	Combustion Heat of Gasoline Determined.....	182
Sulfur Determination in, by Combustion.....	349	Conductometric Titration Apparatus.....	59
Zinc Determination in Cyanide Plating Baths for.....	165	COPPER	
Bromate Determination Iodometrically in Presence of Copper.....	103	Alloys, Silicon Determination in.....	30
Bromine, Unsaturation of Olefins Determined by Substitution with.....	617	Carbon Determination in, by Low-Pressure Combustion.....	24
BRONZE		-Chromium Oxide Catalyst, Preparation and Reclamation.....	44
Copper Determination in, with Salicylimines.....	750	Cupric Ion Determination with 8-Hydroxyquinoline.....	38
Nickel Determination in, by Colorimetric Method.....	354	Determination in Naphthenate of Copper.....	70
Silicon Determination in Manganese Bronzes.....	309	Determination in Presence of Oxidizing Agents.....	10
Sulfur Determination in, by Combustion.....	349	Determination in Steel and Cast Iron. Colorimetric Procedure for Alloy Steels.....	80
Tin Microdetermination with Silicomolybdate in Manganese Bronze.....	269	Group. Polarographic Analysis with Electrolytic Separations.....	14
Brucine Citrate for Bismuth Microdetection.....	761	Microdetermination in Zinc Alloys.....	71
Bubbler of Fritted Glass.....	538	Precipitation and Determination with Salicylimines.....	75
BUNA S (GR-S)		Separation from Tin in Electrodeposition Apparatus.....	53
Analysis, Compounded with Natural Rubber. (Correction, 486).....	9	Copper Perchlorate Standard Solutions, Stability of.....	38
Curing Rate Determination.....	15	Coriander Seed Oil, $\alpha$ -Dicarbonyl Detection in.....	62
<i>o</i> -Xylene Determination in Recycle Styrene.....	751	CORN	
BURETS		Grain, Phytin Phosphorus Determination in.....	38
Calibration for Volume Measurement in Titrimetry.....	50	Lipid Determination in, as Measurement of Maturity.....	70
Micro.....	475	Starch Conversion Products, Refractive Index-Dry Substance Tables for.....	16
for Weighing, Modified Bailey Type of.....	357	Corrections.....	102, 250, 284, 486, 693, 727
Butadiene, Infrared Analysis of.....	422	Correspondence.....	273, 276, 772
Butadiene, Specific Gravity of.....	7	Corrosion, Humidity Cabinet for Tests of.....	394
<i>n</i> -Butane-Isobutane Mixtures Analyzed by Density Method.....	348	COTTON	
Butterfat, Vitamin A and Carotenoids Determined in.....	83, 190	Fabric Dispersions in Cuprammonium, Flow Characteristics of.....	17
Butyl Rubber (GR-I), Curing Rate Determination of.....	562	Molecular Weight of Cellulose.....	35
Butyl Rubber, Sulfur Determination in.....	98	Wax Determination in Fiber by Alcohol Extraction.....	74
2,3-Butylene Glycol Determination in Fermentations.....	626	Cottonseed. Gossypol Determination by Spectrophotometric Method.....	56
CABBAGE, Dehydrated, Sulfur Dioxide Determination in.....	153	Coumarin Determination in Flavoring Extracts.....	50
CADMIUM		<i>o</i> -Cresol Determination in Phenol by Cloud Point (Correspondence).....	72
Microdetermination in Biological Material. Spectrographic, Polarographic, and Colorimetric Methods.....	333	Cresols, Ortho-, Meta-, Para-, Qualitative Tests for Phenol and.....	37
Microdetermination in Zinc Alloys.....	71	Crucible for Filtration, Washing of.....	277, 539
Polarographic Analysis of Copper Group.....	147	Crucible Holder for Rubber Extraction Apparatus.....	72
Selective Spot Reactions for.....	141	Crystalline Materials Determined by X-Ray Diffraction.....	95
Calcium Determination in Magnesium by Spectrographic Method.....	455	Crystallization. Microapparatus for Recrystallization.....	413, 478
Calcium Microdetermination as Oxalate by Titration with Ammonium Hexanitratocerate.....	762	Cuprammonium, Flow Characteristics of Dispersions of Cotton and Regenerated Rayon Fabrics in.....	17
Camphor Determination in Spirit of Camphor by Refractive Index and Specific Gravity.....	168	Cupric Ion. See Copper.....	
Capillary Tube, Funnel for Filling of.....	343	Cupriethylene Diamine as Solvent in Determination of Cellulose Viscosity.....	104
Carbinols, Methyl-, Differentiated from Methyl Ketones.....	362	Cyanide Brass-Plating Baths, Zinc Determination in.....	165
Carbon, Determination by Low-Pressure Combustion.....	242, 248, 634	Cyanide Determination as Hydrocyanic Acid, Especially in Coke-Oven Gas.....	550
Carbon, in Vulcanized Rubber Studied with Electron Microscope.....	642	Cyanogen Bromide in Determination of Nicotine and Nornicotine.....	86
Carbon Black, Pigment Determination in Paints Containing.....	364	<i>p</i> -Cymene, $\alpha$ , $p$ -Dimethylstyrene Determination in Presence of.....	20
Carbon Dioxide Determination in Water.....	315	DEHYDRATED Foods and Feeds.....	153, 513, 628, 632, 638
Carbon Dioxide Generator for Dumas Nitrogen Micromethod.....	344	Demerol (Ethyl-1-methyl-4-phenylpiperidine-4-carboxylate), Microidentification of.....	408
Carbon Disulfide Determination in Air (Correction).....	250	Density. See Specific Gravity.....	
Carbon Monoxide, Iodine Microdetermination in Oxidation of, by Iodine Pentoxide.....	64	DETERGENTS	
Carbonyl Group, Color Test for.....	110	Film Determination on Transparent Surfaces.....	251, 253
Carborundum as Carrier for Copper-Chromium Oxide Catalyst.....	441	in Oxygen-Capacity Determination by Van Slyke Method.....	581
Carius Digestion, Bomb Furnace for.....	308	-Soap Mixtures Analyzed in Bar Form.....	239
CAROTENE DETERMINATION			
in Alfalfa.....	184, 513		
Effect of $\beta$ -Carotene Isomerization on Absorption at 326 Millimicrons.....	328		



Deuterium Oxide Microdetermination, Falling Drop Apparatus for.....	412
Devarda's Alloy. <i>See</i> Alloys.....	
Diacetyl Determination in Mixtures with Ketones.....	469
Dialysis Cell for Microdetermination of Diffusible Components in Blood Plasma.....	136
$\alpha$ -Dicarbonyl Detection in Volatile Essential Oils.....	621
Dichlorodifluoromethane. <i>See</i> Freon-12.....	
Dichromate. <i>See</i> Potassium Dichromate.....	
Diesel Distillate Fuels, Gum Content of.....	710
1-Diethyl-4-aminopentane Purification and Determination.....	431
Diffraction. <i>See</i> X-Rays.....	
Diffusers for Gas, Made of Alundum.....	680
<i>o</i> -Dibydroxybenzene Derivative, Iron Determination with.....	111
Dimethylbenzylammonium Hydroxide. <i>See</i> Triton F.....	
Dimethylglyoxime, Recommended Specification for.....	281
$\alpha$ , $p$ -Dimethylstyrene Determination in Presence of <i>p</i> -Methylstyrene, Styrene, and <i>p</i> -Cymene.....	20
2,4-Dinitroanisole Determination.....	325, 326
Disodium-1,2-dihydroxybenzene-3,5-disulfonate in Iron Determination.....	111
Dispenser for Carr-Price Reagent.....	241
<b>DISTILLATION</b> .....	
Apparatus for Gasoline Analysis.....	525
Bumping Prevention Device.....	722
Column of General Utility.....	639
Flask of General Applicability.....	399
Head for Vacuum Distilling.....	374
under High Vacuum, Ground-Glass Joint for.....	94
Moisture Determination by, Volume Reading Facilitated in.....	720
Molecular Fractionating Still.....	529
Receiver.....	537
Reflux Take-off with Constant Control.....	454
Safety Cap for Glass Equipment.....	276
Dolomite, Magnesia Determination in.....	313
Dropping Funnel of Constant-Rate Type.....	418
Dumas Method. <i>See</i> Nitrogen.....	
Dyes, Azo-2-naphthol Unsulfonated, Identification of.....	755
Dyes for Textile-Finish and Fiber Identification.....	274
<b>EGGS</b> .....	
Asbing Procedure for Liquid White.....	417
Dehydrated, Vitamin A Determination in.....	632
Iron Determination in, by <i>o</i> -Phenanthroline Method.....	317
Moisture Determination in Whole Powder of.....	628
Electrode, Glass, for Vitamin Microbiological Assay.....	205
Electrode, Hydrogen, Circulating Device for Use with.....	585
Electrodeposition, Automatic Control Apparatus with Graded Cathode Potential.....	532
Electron Microscope. <i>See</i> Microscope.....	
<b>EMULSIONS</b> .....	
Acidity Determination by Potentiometric Method.....	219
Extractor for Mixtures Tending to Emulsify.....	62
Oil Determination in, by Fluorocolorimetric Method.....	331
Polymerization of Synthetic Rubber in 10-Gram Systems.....	1
Waring Blender Adapted to Continuous Preparation of.....	717
Enamel Baking Control Methods.....	599
( <i>See also</i> Paint.).....	
Equipment, New.....	143, 278, 350, 418, 539, 652, 722
Ergosterol Determination by Colorimetric Method.....	495
Ester Saponification by Semimicroprocedure.....	410
<b>ETHANOL</b> .....	
Determination in Spirit of Camphor by Refractive Index and Specific Gravity.....	168
-Ethyl Ether-Benzene-Water Solutions Analyzed.....	432
Wax Determination in Cotton Fiber by Extraction with.....	745
Ethanolamine Hydrolysis for Determination of Methyl Bromide.....	538
Ethyl Acetate Determination in Presence of Acetaldehyde.....	734
Ethylbis-2,4-dinitrophenyl Acetate as pH Indicator.....	53
Ethyl Ether-Benzene-Ethanol-Water Solutions Analyzed.....	432
Ethyl-1-methyl-4-phenylpiperidine-4-carboxylate. <i>See</i> Demerol.....	
Evaporation of Standard Solutions, (Predictable) Concentration Resulting from.....	349
Evaporator, Constant-Level, for Determination of Total Solids.....	120
Evaporator, Rapid-Circulating, of Glass.....	754
<b>EXTRACTORS</b> .....	
Continuous Versatile Type of.....	332
Large Glass Apparatus.....	61
Liquid-Liquid.....	62, 180, 473
for Solids, Large Continuous Type of.....	472
Spray Column.....	528
<b>FABRICS, Cotton and Regenerated Rayon. Flow Characteristics of Dispersions in Cuprammonium.....</b>	172
<b>FATS</b> .....	
Acidity Determination in, by Potentiometric Method.....	219
Ascorbyl Ester Determination in.....	464
Butter, Vitamin A and Carotenoids Determined in.....	83, 190
Gallic Acid Determination as Antioxidant in.....	427
Margarine, Vitamin A Determination in.....	358
Unsaturated, Ultraviolet Absorption Spectra of.....	385
<b>FATTY ACIDS</b> .....	
Amines, Primary Aliphatic, of High Molecular Weight Determined in.....	459
Conjugation, Absorption Spectra Analysis of.....	77
Esters of <i>l</i> -Ascorbic and <i>d</i> -Isoascorbic Acids Determined in Fats and Oils.....	464
Methyl Esters, Vapor Pressures of.....	605
Unsaturated, Ultraviolet Absorption Spectra of.....	385
Feeds, Sulfur Determination in.....	630
<b>FERMENTATIONS</b> .....	
2,3-Butylene Glycol Determination in.....	626
Citric Acid Determination in Media for.....	515
for <i>d</i> -Galactose Determination by Quantitative Method.....	28
Ferric Ion. <i>See</i> Iron.....	
<b>FERTILIZER</b> .....	
Hygroscopicity Determination.....	367
Moisture Absorption Determined by Accelerated Method.....	487
Potassium Determination. Removal of Ammonia and Organic Matter without Ignition.....	383
Fiber of Cotton, Wax Determination in, by Alcohol Extraction.....	745
Fiber Identification Stains.....	274

Film Permeability to Gases, Apparatus for Measurement of.....	58
Films, Organic, Water Vapor Permeability of.....	686
Filter-Cake Surface Renewal in Vacuum Filtrations.....	365
Filter Crucible, Washing of.....	277, 539
Fischer, Karl, Reagent for Analysis of Acetylsulfanilyl Chloride.....	517
<b>FISH</b> .....	
Dehydrated, Instrument for Measuring Changes in Texture of.....	638
Liver Oils, Vitamin A Potency of.....	126, 288, 436, 509
Liver Oils. Vitamin D Estimation with Antimony Trichloride.....	179
Spoilage Determination in.....	490, 593
Flame of Acetylene-Air for Spectrochemical Analysis.....	728
Flame, Microtorch for.....	142
<b>FLASKS</b> .....	
for Distillation, of General Applicability.....	399
Funnel for Standard Taper Type of.....	293
Support for Kjeldahl Type of.....	324
Support on Steam and Water Baths.....	332
Flow of Cellulose-Cuprammonium Dispersions.....	172
Flow, Liquid Delivery at Low and Constant Rates of.....	471
Flowmeter of Positive Displacement Type.....	303
Fluoride Determination in Foods and Biological Material.....	457
<b>FOOD</b> .....	
Dehydrated, Sulfur Dioxide Determination in.....	153
Fluoride Determination in Toxic Quantities.....	457
Iron Microdetermination in.....	646
Packaging Materials, Apparatus for Measuring Rate of Gas Penetration through.....	586
Protein Nutritive Value Determined in.....	696
Proteinaceous, Spoilage Determined in, Particularly Fish.....	490
( <i>See also</i> individual food.).....	
Formaldehyde Determination in Presence of Acrolein and Other Aldehydes. (Correction, 693).....	496
<b>FRACTIONATION</b> .....	
of Asphalts by Solvent Separation.....	294
Column for Distilled Water of High Purity.....	748
Column for Gases.....	131
Columns, Precision Head for.....	584
Pressure Control in, at Low Pressure and Temperature.....	40
( <i>See also</i> Distillation.).....	
Freeze Tests, Alternating Current Solenoid for.....	588
<b>FREON-12 (DICHLORODIFLUOROMETHANE)</b> .....	
Insecticide Concentration Increased in Aerosol-Producing Solution of.....	355
Noncondensables Determined in.....	681
Pyrethrum Solubility Determined in.....	453
<b>FUNNELS</b> .....	
for Capillary Tube Filling.....	343
Dropping Type of.....	418, 641
for Standard Taper Flasks.....	293
for Weighing.....	133
<b>FURNACE</b> .....	
Determination. Iodine Method for Hydrolyzed Wood Liquors.....	319
in Hydrocarbon Solution-Temperature Determination.....	107
-Pentose Method for Pectin Microdetermination in Biological Materials.....	74
Furnace Bomb for Carius Digestion.....	308
Furnace with Controlled Atmosphere for Induction Melting.....	302
<b><i>d</i>-GALACTOSE</b> Determination by Selective Fermentation.....	28
Gallic Acid Determination as Antioxidant in Fats and Oils.....	427
<b>GASES</b> .....	
Coke-Oven, Hydrocyanic Acid Determination in.....	550
Collection at Low Pressures with Automatic Pump.....	592
Diffusers Made of Alundum.....	680
Fractionating Column.....	131
Hydrogen Determination in Exhaust from Catalytic Dehydrogenation Unit.....	719
Nitric Oxide Microdetermination in.....	766
Noncondensables Determined in.....	681
Penetration through Food-Packaging Materials, Apparatus for Measuring Rate of.....	586
Permeability of Films of Low Permeability to, Apparatus for Measurement of.....	58
( <i>See also</i> individual gas.).....	
<b>GASOLINE</b> .....	
Analysis in Automatic Distillation Apparatus.....	525
Combustion Heat Determined for.....	182
Tetraethyllead Determination in.....	4
<b>GERMANIUM DETERMINATION</b> .....	
by Gravimetric and Volumetric Methods.....	322
as Molybdiagermanic Acid.....	128
in Steel by Gravimetric Method.....	311
Gibbs Method. <i>See</i> Phenols.....	
<b>GLASS</b> .....	
Bubbler of Fritted Glass.....	538
Detergent-Hard Water Film Determination on.....	251, 253
Distillation Equipment, Safety Cap for.....	276
Electrode for Titrating Microbiological Vitamin Assays.....	205
Evaporator of Rapid Circulating Type.....	754
Extraction Apparatus of Large Size.....	61
Joint for High-Vacuum Distillation.....	94
Midget Impinger Unit.....	346
Plastic Tubing Joined to.....	275
Glucose, Maltose Determination in Presence of.....	582
Gossypol Determination in Cottonseed and Meal by Spectrophotometric Method.....	566
GR-I. <i>See</i> Butyl Rubber.....	
GR-S. <i>See</i> Buna S.....	
Grinding Plant Samples, Mineral Contamination from.....	202
Guayule, Staining Rubber in Ground or Milled Tissues of.....	480
Gum Content of Distillate Diesel Fuels.....	710
Gutzeit Spot Filtration for Ultramicrodetermination of Arsenic.....	400
<b>HALLETT, L. T., Appointed Associate Editor.....</b>	272
Heater, Electric, for Microprocedures and Melting Points.....	134
Hemicellulose. <i>See</i> Cellulose.....	
Humidity Cabinet for Corrosion Testing.....	394
Hydriodic Acid, Recommended Specification for.....	281
<b>HYDROCARBONS</b> .....	
Analysis by Sulfuric Acid Extraction. (Correction, 727).....	558
Liquids Purified as Solvents for Absorption Spectroscopy.....	556
Olefin Unsaturation Determined by Bromine Substitution.....	617



Physical Methods of Analysis.....	723	Meal, Soybean, Detection of Inadequate Heat Treatment of.....	640
Pycnometer for Volatile Liquids.....	55	Melting Point, Electric Heater for.....	134
Solution Temperatures Determined with Furfural.....	107	Mercuric Oxide (Yellow), Recommended Specification for.....	281
Standard Samples of Bureau of Standards.....	273	METAL	
Stopcock Lubricant Unaffected by.....	415	Furnace for Melting of.....	302
Thinners, Evaporation Indices of.....	690	Paint Pigment Evaluation.....	234
Hydrocyanic Acid Determination, Especially in Coke-Oven Gas.....	550	Phosphomolybdic Acid Color Reaction with.....	637
Hydrogen Determination in Gaseous Exhaust with Thermal-Conduc-		Polarographic Analysis. Copper Group.....	147
tivity Apparatus.....	719	Spectrographic Qualitative Analysis.....	675
Hydrogen Fluoride, Anhydrous, Sampling and Analysis of.....	419, 483	(See also Alloys and individual metal.)	
Hydrogen-Ion Concentration Indicator, Ethylbis-2,4-dinitrophenyl		Meteoric Iron. See Iron.	
Acetate.....	53	Methoxyl Determination in Pectin. Errors in Zeisel Method Due to	
Hydrogen Peroxide, Nitrate Removal from.....	181	Retained Alcohol.....	523
Hydrogenation Reaction Mixtures, Catalyst Separation from.....	637	Methyl Bromide Determination by Ethanamine Hydrolysis.....	538
Hydroxylamine Hydrochloride Color Test for Carbonyl Group.....	110	Methyl Esters of Fatty Acids Identified by Vapor Pressure Curves.....	605
8-Hydroxyquinoline as Analytical Reagent.....	387	Methyl Groups Linked by Carbons, Determination of.....	434
8-Hydroxyquinoline in Magnesium Microdetermination.....	596	Methyl Ketone Differentiation from Methylcarbinols.....	362
		Methyl Vinyl Ketone Determination in Mixtures with Methylvinyl-	
		carbinol and Methyl Ethyl Ketone.....	469
		Methylcarbinol Differentiation from Methyl Ketones.....	362
		p-Methylstyrene, Determination of $\alpha$ ,p-Dimethylstyrene in Presence	
		of.....	20
		Microchemical Balances. Errors of Kuhlmann Balance.....	258
		Microchemistry.....	63, 134, 202, 258, 333, 400, 475, 536, 593, 642
		Microprocedures, Electric Heater for.....	134
		Microscope, Electron, Carbon in Vulcanized Rubber Studied with.....	642
		Milk, Vitamin A and Carotene Determined in.....	101
		Minerals, Lithium Determination in.....	712
		Minerals as Plant-Sample Contamination after Grinding.....	202
		Moisture. See Water.	
		Molecular Fractionating Still.....	529
		Molybdenum, Microdetermination in Plants and Soils.....	137
		Molybdenum Blue Reaction Studied.....	466
		Molybdenum Blue for Silica Determination in Aluminous Materials.....	705
		Molybdigermanic Acid, Germanium Determination as.....	128
		Molybdivanadophosphoric Acid, Colorimetric Determination of Phos-	
		phorus as.....	379
		Monel Metal Pouring Plate for Silica Fusions.....	590
		Mucilages from Plants, d-Galactose Determination by Selective Fer-	
		mentation of.....	28
		Muscle of Beef, Thiamine Determination in.....	116
		Muscle, Vitamin A Assay by Destructive Irradiation of.....	288
		Mustard Gas Determined in Compact Field Apparatus.....	255
		NAPHTHAS. Evaporation Indices of Hydrocarbon Thinners.....	690
		Naphthenates of Copper and Zinc, Determination of Copper and Zinc	
		in.....	703
		2-Naphthol, Azo-, Dyes, Unsulfonated, Identification of.....	755
		NICKEL	
		Determination in Bronze by Colorimetric Method.....	354
		Determination in Steel by Colorimetric Method.....	375
		Precipitation with Salicylimines.....	750
		Raney, as Catalyst. Separation from Hydrogenation Reaction	
		Mixtures.....	637
		Nicotine Determination in Mixture with Nicotinic.....	86
		NITRATE	
		Determination in Soil and Plant Extracts by Photometric Method..	446
		Microdetermination by Devarda Method.....	764
		Removal from Hydrogen Peroxide of 30% Strength.....	181
		Nitric Oxide Microdetermination in Gases.....	766
		Nitric Oxide Microdetermination with Solid Reagents.....	341
		Nitrite Determination in Soil and Plant Extracts by Photometric	
		Method.....	446
		Nitrocellulose, Sulfate Determination in.....	391
		NITROGEN	
		Determination in Plant Material by Photometric Method.....	121
		Determination in Soil and Plant Extracts as Nitrate, Nitrite, and	
		Ammonium.....	446
		Dumas Micromethod, Carbon Dioxide Generator for.....	344
		Extraction from Vegetables, Free of Carbohydrate.....	609
		Kjeldahl Flasks, Support for.....	324
		Nitrogen Dioxide Determination by Cerate Oxidimetry.....	366
		Nitroso R Salt Method for Microdetermination of Iron Improved...	276
		Nomograph for Weight Correction to Vacuum.....	275
		Nornicotine Determination in Mixture with Nicotine.....	86
		Nornicotine Identification in Tobacco.....	377
		OAT Grain Contamination from Grinding.....	202
		OILS	
		Acidity Determination by Potentiometric Method.....	219
		Ascorbyl Ester Determination in.....	446
		of Dark Color, Ethylbis-2,4-dinitrophenyl Acetate as pH Indicator	
		for.....	53
		Determination in Blends and Emulsions by Fluorocolorimetric	
		Method.....	331
		Drying and Semidrying. Analysis When Heat-Bodied.....	90
		Essential Volatile, $\alpha$ -Dicarbonyl Detection in.....	621
		Fish Liver, Vitamin Potency of.....	126, 179, 436, 509
		Gallic Acid Determination as Antioxidant in.....	427
		for Quenching Aluminum Alloy Castings, Spectrophotometric Study	
		of Oxidation of.....	740
		Sesame, Determination of Sesamin in.....	166
		Tung Estimation as Adulterant in Other Oils.....	511
		Vitamin A Assay by Destructive Irradiation.....	288
		Olefin Unsaturation Determined by Bromine Substitution.....	617
		Orange Peel Oil, $\alpha$ -Dicarbonyl Detection in.....	621
		ORGANIC COMPOUNDS	
		Acid Microidentification by Partition Method.....	479
		Bomb Furnace for Carius Digestion.....	308
		Vapors, Stopcock Lubricants for Use with.....	415
		ORGANIC MATTER	
		Arsenic Ultramicrodetermination in, by Gutzeit Spot Filtration...	400
		Phosphorus Microdetermination in, by Rapid Digestion.....	345
		(See also Biological Material.)	
		Osmium Trace Determination by Colorimetric Method.....	342
		Osmometry of High-Polymer Solutions.....	520
		Oxidation-Reduction Indicators, Substituted 1,10-Phenanthroline	
		Complexes as.....	580
		OXYGEN	
		Capacity Determination in Blood, Using Detergents.....	581
IMPINGER, Glass Midget Type of.....	346		
Infrared. See Spectrometry.			
INSECTICIDES			
Aerosol-Producing Solution in Freon-12, Increasing Concentration			
of.....	355		
Arsenic Semimicrodetermination in.....	414		
2,4-Dinitroanisole Determination.....	325, 326		
Pyrethrum Solubility in Freon Determined.....	453		
Xanthone Spray Residues Analyzed Colorimetrically.....	35		
Iodate Determination Iodometrically in Presence of Copper.....	103		
Iodine for Furfural Determination in Hydrolyzed Wood Liquors.....	319		
Iodine Microdetermination in Oxidation of Carbon Monoxide by			
Iodine Pentoxide.....	64		
Iodine Monochloride in Permanganate Solution Standardization			
against Arsenious Oxide.....	625		
Iodine Number Determination for Tall Oil.....	371		
Iodine Number, Wijs Solution Stabilized for Determination of.....	417		
Iodine Pentoxide Oxidation of Carbon Monoxide, Iodine Microdeter-			
mination in.....	64		
Iodometry, Starch Prepared as Indicator for.....	772		
Ion-Exchange Adsorber, Quantitative Separations with.....	615		
Ion Exchangers in Determination of Pectin and Pectic Acid.....	23		
IRON			
Carbon Determination in, by Low-Pressure Combustion.....	242, 634		
Cast, Copper Determination in, by Electrolytic Method.....	80		
Determination with Dichromate, Using Silver Reductor.....	49		
Determination by o-Phenanthroline Method.....	317		
Ferric, Determined with Disodium-1,2-dihydroxybenzene-3,5-disul-			
fonate.....	111		
Ferric Ion Determination with 8-Hydroxyquinoline.....	387		
Meteoric, Osmium Trace Determination in.....	342		
Microdetermination in Food Products.....	646		
Microdetermination by Nitroso R Salt Method Improved.....	276		
-Phosphorus Stoichiometric Relation in Ferric Phytate.....	389		
Vitamin C Estimation in Presence of Salts of.....	757		
Iron Blue Paint, Pigment Determination in.....	364		
d-Isoascorbic Acid Esters Determined in Fats and Oils.....	464		
Isobutane-n-Butane Mixtures Analyzed by Density Method.....	348		
JOINT of Ground Glass for High-Vacuum Distillations.....	94		
Juniper Berry Oil, $\alpha$ -Dicarbonyl Detection in.....	621		
KETONES, Methyl, Differentiated from Methylcarbinols.....	362		
Ketones, Methyl Vinyl, Determination in Mixtures with Methyl-			
vinylcarbinol and Methyl Ethyl Ketone.....	469		
Kjeldahl. See Nitrogen.			
Kuhlmann Microbalance, Errors of.....	258		
LABORATORY of American Hotel Association.....	539		
Leach Liquors, Colorimetric Procedures for: Aluminum Determi-			
nation, 169; Silica Determination.....	462		
LEAN			
Determination in Gasoline as Tetraethyl.....	4		
Microdetermination in Zinc Alloys.....	71		
Polarographic Analysis of Copper Group.....	147		
Lead Subacetate, Recommended Specification for.....	281		
Leuco Crystal Violet Microdetermination after Oxidation with Benzoyl			
Peroxide.....	336		
Level Control for Liquids by Photoelectric Relay.....	393		
Lewistite Determined in Compact Field Apparatus.....	255		
Limestone, Phosphorus Determination in, by Photometric Method...	553		
Linoleic Acid Conjugation, Absorption Spectra Analysis of.....	77		
Linseed Oil, Heat-Bodied, Analysis of.....	90		
Lipid Determination in Vegetable Matter.....	701		
LIQUIDS			
Delivery at Low and Constant Rates.....	471		
Extractors for.....	62, 180, 473		
Level Control by Photoelectric Relay.....	393		
Volatile, Pycnometer for.....	55		
Lithium Determination in Minerals.....	712		
Lubricants from Petroleum Containing Additives. Acid Determi-			
nation by Potentiometric Method.....	219		
Lubricants for Stopcocks.....	415		
MAGNESIA Determination in Magnesite and Dolomite.....	313		
Magnesite. See preceding item.			
MAGNESIUM			
Alloys, Zinc Determination in.....	256, 460		
Calcium Determination in, by Spectrographic Method.....	455		
Determination with 8-Hydroxyquinoline.....	387		
Microdetermination with 8-Hydroxyquinoline by Polarographic			
Method.....	596		
Maltose Determination in Presence of Glucose.....	582		
Manganese Determination in Large Amounts by Persulfate Method...	560		
Manganese Determination after Oxidation to Tri-Dihydrogen Pyro-			
phosphatomanganate.....	187		
Manganese Sulfate Monohydrate, Recommended Specification for...	281		
Margarine, Vitamin A Determination in.....	358		
Meal, Cottonseed, Gossypol Determination in.....	566		



Determination of Penetration Rate of, into Food-Packaging Materials.....	586	Pouring Plate of Monel Metal for Silica Fusions.....	590
Dissolved, Stable Starch Solution for Determination of.....	369	Powder Diffraction. See X-Rays.	
<b>PAINT</b>		Precipitants, Salicylimines as Organic Type of.....	750
Dry Hiding Power Determination of.....	442	<b>PRESSURE</b>	
Pigment Determination in Carbon Black and Iron Blue Types of...	364	Control in Fractionation at Low Pressure and Temperature.....	40
Pigment Evaluation for Metal Protection.....	234	Fatty Acid Methyl Esters Identified by Vapor Pressure Curves....	605
Thinners. Evaporation Indices of Solvent Naphthas.....	690	Osmotic, Measured for High-Polymer Solutions.....	520
(See also Enamel.)		<b>PROTEIN</b>	
Palladium Separation from Hydrogenation Reaction Mixtures.....	637	Filtration under Vacuum. Device for Renewing Filter-Cake Surface.	365
Pantothenic Acid Determination by Yeast Microbiological Method....	67	in Food, Determination of Nutritive Value of.....	696
Paper Package Coatings, Apparatus for Measuring Gas Permeability		Foodstuffs, Determination of Spoilage in, Particularly Fish.....	490
of.....	58	Pulp. Hemicellulose Determination by Photometric Method.....	429
Peanut Oil, Tung Oil Estimation as Adulterant in.....	511	Pulp from Wood. Molecular Weight of Cellulose.....	351
Pectic Acid Determination in Solution by Electrodeposition.....	23	Pump for Gas Collection Automatically at Low Pressures.....	592
<b>PECTIN</b>		Pycnometer for Volatile Liquids.....	55
Methoxyl Determination in. Errors in Zeisel Method Due to Retained Alcohol.....	523	Pyrethrolone, Determination of Carbon-Linked Methyl Groups in....	434
Microdetermination in Biological Materials.....	74	Pyrethrum Solubility in Freon Determined.....	453
Soluble, Determination by Electrodeposition.....	23	Pyridine Estimation in Mixture with Acetic Acid for Riboflavin Determination.....	583
Penicillin Estimation by Rapid Method.....	451	Pyridine for Manganese Separation from Iron, Chromium, Vanadium, and Cerium.....	187
4-Pentene, Anti-1,5-di-( <i>p</i> -methoxyphenyl)-1-hydroxylamino-3-oximino-, for Tungsten Determination.....	45	<b>QUALITATIVE Analysis, Spectrographic Techniques for.....</b>	675
Pentose-Furfural Method for Pectin Microdetermination in Biological Materials.....	74	Quantitative Analysis, Spectrographic Emission Equipment for. Proposed Minimum Requirements.....	670
Perchloric Acid, Recommended Specification for.....	281	Quantitative Analysis, Spectrographic Techniques for.....	653
Perilla Oil, Heat-Bodied, Analysis of.....	90	Quenching Oil. See Oils.	
Permanganate Determination Iodometrically in Presence of Copper...	103	Quinacrine Hydrochloride, Amino Side-Chain Analysis of.....	431
Permanganate Solution Standardization against Arsenious Oxide with Iodine Monochloride.....	625	Quinine as Standard in Thiamine Determination. Effect of Temperature and Dissolved Oxygen.....	572
Permeability of Film Material to Gas, Apparatus for Measurement of.	58	<b>RACK for Semimicrotitration.....</b>	346
Permeability of Organic Films to Water Vapor.....	686	Raney Nickel Separation from Hydrogenation Reaction Mixtures....	637
<b>PETROLEUM</b>		Rapeseed Oil, Tung Oil Estimation as Adulterant in.....	511
Distillate Diesel Fuels, Gum Content of.....	710	<b>RAYON</b>	
Fractions, Furfural Solution Temperatures of.....	107	Hemicellulose Determination by Photometric Method.....	429
Hydrocarbon Analysis by Sulfuric Acid Extraction. (Correction, 727).....	558	Molecular Weight of Cellulose.....	351
Hydrocarbons. Physical Methods of Analysis.....	723	Regenerated Fabric Dispersions in Cuprammonium, Flow Characteristics of.....	172
Oil-Soluble Sodium Sulfonates Analyzed by Adsorption.....	25	Reading Scale of Microbalance, Device for Projecting Image of.....	645
(See also Gasoline and Lubricants.)		Reagent Analytical Chemicals, Recommended Specifications for....	281
Pharmaceuticals, Thiamine Microdetermination in.....	476	Reducing Agents, Phosphomolybdic Acid Color Reaction with.....	637
1,10-Phenanthroline Ferrous Complexes as Oxidation-Reduction Indicators.....	580	Reduction-Oxidation Indicators, Substituted 1,10-Phenanthroline Ferrous Complexes as.....	580
<i>o</i> -Phenanthroline Method for Iron Determination.....	317	Reflux. See Distillation.	
<b>PHENOLS</b>		Refractive Index, Spirit of Camphor Analysis by Specific Gravity and.	168
Alkylated, Phenyl Isocyanate Derivatives of. Melting Points and X-Ray Diffraction.....	304	Refractive Index of Starch Conversion Products.....	161
<i>o</i> -Cresol Determination in, by Cloud Point (Correspondence).....	772	Reservoir for Hot Distilled Water.....	201
Determination in Dilute Solutions by Gibbs Method.....	694	<b>RESINS</b>	
Qualitative Tests for <i>o</i> -, <i>m</i> -, <i>p</i> -Cresols and.....	37	Acidity Determination by Potentiometric Method.....	219
Phenyl Isocyanate Derivatives of Alkylated Phenols. Melting Points and X-Ray Diffraction.....	304	Alkyd Oil-Modified, Phthalic Anhydride Determination in.....	198, 200
Phosphates, Silica Determination in Condensed Steam in Presence of.	574	Identification by Systematic Procedure.....	541
Phosphomolybdic Acid Color Reaction Studied Qualitatively.....	637	(See also Plastics.)	
<b>PHOSPHORUS</b>		Riboflavin Determination, Pyridine Content of Pyridine-Acetic Acid Mixture Used in.....	583
Determination in Limestone by Photometric Method.....	553	Rotenone Color Tests Not Specific.....	277
Determination by Molybdenum Blue Reaction.....	466	<b>RUBBER</b>	
Determination as Molybdivanadophosphoric Acid.....	379	Analysis, Compounded with Synthetic Rubber. (Correction, 486)...	9
Determination as Phytate. Iron-Phosphorus Relation in Ferric Phytate.....	389	Carbon-Reinforced, Electron Microscope Study of.....	642
Determination in Plant Material by Photometric Method.....	121	Connections to Glass Tubes.....	348
Microdetermination by Rapid Digestion.....	345	Curing Rate Determination.....	15, 562
Phosphorus Pentoxide, Recommended Specification for.....	281	Extraction Apparatus, Crucible Holder for.....	721
<b>PHOTOMETRY</b>		Extraction Apparatus, Solvent-Recovery Take-Off Device for.....	722
Analysis of Errors at High and Low Absorptions.....	123	Freeze Tests, Alternating Current Solenoid for.....	588
Liquid Level Automatic Control in.....	393	Identification of Elastomers by Rapid Method.....	424
Photometer for Carbon Disulfide Determination in Air (Correction).....	250	Staining in Ground or Milled Plant Tissues.....	480
Photometer for Film Determination in Detergent Processes.....	251	<b>RUBBER, SYNTHETIC</b>	
Phthalate Determination.....	416	Analysis, Compounded with Natural Rubber. (Correction, 486)...	9
Phthalic Anhydride Determination in Oil-Modified Alkyd Resins....	198, 200	Buna S. <i>o</i> -Xylene Determination in Recycle Styrene.....	751
Phytin Phosphorus Determination.....	389	Butadiene Specific Gravity.....	7
Pigments. See Paint.		Butyl, Curing Rate Determination of.....	562
<b>PLANT MATERIAL</b>		Butyl, Sulfur Determination in.....	98
Ascorbic Acid Microdetermination in Large Numbers of Samples....	649	Carbon-Reinforced, Electron Microscope Study of.....	642
Carotene Determination in, by Chromatographic Method.....	184	Curing Rate Determination.....	15
Chlorophyll, Carotene, and Xanthophyll Determination in.....	438	Emulsion Polymerization in 10-Gram Systems.....	1
Magnesium Microdetermination in, with 8-Hydroxyquinoline.....	596	Freeze Tests, Alternating Current Solenoid for.....	588
Mineral Contamination in Grinding of.....	202	Identification of Natural Rubber and.....	424
Molybdenum Microdetermination in.....	137	<b>SAFETY Cap for Glass Distillation Equipment.....</b>	276
Nitrate Determination, Purification of 30% Hydrogen Peroxide for.	181	Salicylimines as Organic Precipitants.....	750
Nitrate, Nitrite, and Ammonium Nitrogen Determination in Extracts of.....	446	Saponification of Esters by Semimicroprocedure.....	410
Nitrogen, Phosphorus, and Potassium Determination in.....	121	Sesame Oil, Sesamin Determination in.....	166
Staining Rubber in, When Ground or Milled.....	480	Sesame Oil, Tung Oil Estimation as Adulterant in.....	511
Starch Determination in, by Polarimetric Method.....	736	Sesamin Determination in Sesame Oil.....	166
Plasma. See Blood.		Shark Liver, Extraction of Oil and Vitamin A from.....	126
Plasticizer Determination in Cellulose Esters.....	93	<b>SILICA</b>	
<b>PLASTICS</b>		Determination in Aluminous Materials by Molybdenum Blue Reaction.....	705
Films, Water Vapor Permeability of.....	686	Determination by Colorimetric Methods.....	462
Identification by Systematic Procedure.....	541	Determination, Dissolved in Water.....	612
Tubing Joined to Glass.....	275	Determination in Steam in Presence of Phosphates.....	574
(See also Resins.)		Fusions, Monel Metal Pouring Plate for.....	590
Platinum Separation from Hydrogenation Reaction Mixtures.....	637	Silicomolybdate in Tin Microdetermination by Colorimetric Method.	269
Polarographic Cell for Rapid Analysis.....	329	Silicon Determination in Aluminum.....	705
Polarographic Metal Analysis. Copper Group.....	147	Silicon Determination in Copper-Base Alloys by Photometric Method.	309
Polymerization by Emulsion of Synthetic Rubber in 10-Gram Systems.	1	Silver Reductor in Iron Determination with Dichromate.....	49
Polymers. Acidity Determination by Potentiometric Method.....	219	Siphon for Timing.....	592
Polymers. Osmometry of High-Polymer Solutions.....	520	Sirup from Corn, Refractive Index-Dry Substance Tables for.....	161
Potassium Determination in Fertilizer Mixtures. Removal of Ammonia and Organic Matter without Ignition.....	383	Soap-Detergent Mixtures Analyzed in Bar Form.....	239
Potassium Determination in Plant Material by Photometric Method.	121	Soap-Oil Mixtures Analyzed by Adsorption of Sodium Sulfonates....	25
Potassium Dichromate Aqueous Solutions, Color of.....	42	Sodium Determination in Potassium Hydroxide.....	157
Potassium Dichromate in Iron Determination, Using Silver Reductor.	49	Sodium Aluminate, Silica Determination in Solutions of.....	705
Potassium Ferricyanide and Ferrocyanide. See Cyanide.		Sodium Hypochlorite in Titrimetric Microanalysis.....	206
Potassium Hydroxide, Sodium Determination in.....	157	Sodium Phosphate (Dibasic, Heptahydrate), Recommended Specification for.....	281
Potassium Iodate Standard Solutions, Stability of.....	38	Sodium Sulfonates Soluble in Petroleum Oil, Analyzed by Adsorption.	25
Potassium Permanganate Standardization against Arsenious Oxide with Iodine Monochloride.....	625	Sodium Thiosulfate Standardization for Titrations.....	38
Potato, Dehydrated, Sulfur Dioxide Determination in.....	153	Soil, Molybdenum Microdetermination in.....	137
Potato, Sweet, Starch Determination in Products from.....	736	Soil, Nitrate, Nitrite, and Ammonium Nitrogen Determination in Extracts of.....	449
Potentiometric Acidity Determination in Highly Colored Materials.			
Petroleum Lubricants Containing Additives.....	219		



Solenoid of Alternating Current Type for Freeze Tests.....	588	Turpentine, Destructively Distilled. Detection in Other Turpentines by Aniline Point.....	603
<b>SOLIDS</b>		<b>ULTRAVIOLET.</b> See Spectrometry.	
Analysis by Powder Diffraction.....	209	<b>VALVE</b> of Constant-Level Float Type.....	201
Extractor of Large Continuous Type.....	472	Van Slyke Method. See Blood.	
Microapparatus for Purification of.....	478	Vanilla Extracts, Vanillin and Coumarin Determined in.....	505
Total Apparatus for Determination in Water.....	120	Vanillin Determination in Flavoring Extracts. See preceding item.	
Solubility Microdetermination, Apparatus for.....	413	Vegetables, Amino Acid Analysis of. Carbohydrate-Free Extraction of Nitrogen.....	609
Solution Concentration (Predictable) Owing to Evaporation.....	349	Vegetables, Lipid Determination in, as Measurement of Maturity....	701
Solvent Hydrocarbons Purified for Absorption Spectroscopy.....	556	Viscosity of Cellulose, Precise Method for.....	104
Solvent Recovery by Take-Off Device for Rubber Extraction Appa- ratus.....	722	Viscosity Measurement. Calibrated Master Viscometers.....	708
<b>SOYBEAN</b>		<b>VITAMINS</b>	
Lipid Determination in, as Measurement of Maturity.....	701	A, Alcohol and Ester Forms Separated by Solvent Extraction and Chromatographic Methods.....	509
Meal, Detection of Inadequate Heat Treatment of.....	340	A, and Carotene Determined in Milk.....	101
Oil, Heat-Bodied, Analysis of.....	90	A, and Carotenoids Determined in Butterfat.....	83, 190
Specific Gravity Determinations. Pycnometer for Volatile Liquids....	55	A, Determination in Dehydrated Eggs.....	632
Specific Gravity, Spirit of Camphor Analysis by Refractive Index and.	168	A, Determination in Fish Liver Oils by Spectroscopic Method....	436
Specifications for Reagent Analytical Chemicals.....	281	A, Determination in Margarine by Spectrophotometric Method....	358
<b>SPECTROMETRY</b>		A, Reagent Dispenser for Determination of.....	241
Absorption Solvents Purified for.....	556	A, Shark Liver Analysis for Oil and.....	126
Acetylene-Air Flame for.....	728	A, Spectrophotometric Assay by Destructive Irradiation.....	288
Emission Equipment for Quantitative Analysis. Proposed Mini- mum Requirements.....	670	Ascorbic Acid Microdetermination in Plant Samples.....	649
Infrared Analysis of Butadiene.....	422	Ascorbyl Esters Determined in Fats and Oils.....	464
Qualitative Techniques.....	675	Assays, Electrode for Microtitration of.....	205
Quantitative Techniques.....	653	C, Estimation in Presence of Iron Salts.....	757
Ultraviolet Absorption of Unsaturated Fats and Fatty Acids.....	385	Carotene, Chlorophyll, and Xanthophyll Determination in Plants....	438
Spoilage Determination in Protein Foodstuffs and Fish.....	490	Carotene Determination in Alfalfa.....	184, 513
Spoilage in Fish, Volatile Base Microdetermination as Index of.....	593	$\beta$ -Carotene Isomerization as Affecting Absorption at 326 Milli- microns.....	328
Spot Reaction for Cadmium.....	141	D, Antimony Trichloride Reaction of.....	179
Spray Residues of Xanthone Analyzed Colorimetrically.....	35	Ergosterol Determination by Colorimetric Method.....	495
<b>STARCH</b>		Pantothenic Acid, Yeast Microbiological Determination of.....	67
Conversion Products, Refractive Index-Dry Substance Tables for..	161	Riboflavin Determination, Estimation of Pyridine in Acetic Acid- Pyridine Mixture for.....	583
Determination in Sweet Potato Products and Other Plant Materials.	736	Thiamine Determinations.....	116, 476, 572, 576
Ground as Indicator in Iodometry.....	772	Volume Measured Precisely in Titrimetric Analysis.....	50
Solution Stabilized for Dissolved Oxygen Determinations.....	369	Volume Reading Facilitated in Moisture Determinations by Dis- tillation.....	720
Steam, Silica Determination in.....	574, 612	<b>WARING</b> Blender for Continuous Emulsification.....	717
<b>STEEL</b>		Wastes, Industrial. Phenol Determination in Dilute Solutions.....	694
Carbon Determination in, by Low-Pressure Combustion....	242, 248, 634	<b>WATER</b>	
Chromium Determination in, by Colorimetric Method.....	507	Absorption Determined by Accelerated Method.....	487
Copper Determination in, by Colorimetric Procedure.....	80	Alkalinity Indicator for Boiler Feedwater (Correspondence).....	273
Germanium Determination in, by Gravimetric Method.....	311	Carbon Dioxide Determination in.....	315
Nickel Determination in, by Colorimetric Method.....	375	-Detergent Film Determination on Transparent Surfaces.....	251, 253
Phosphorus Determination in, as Molybdivanadophosphoric Acid....	379	Determination by Distillation, Volume Reading Facilitated in....	720
Tin Microdetermination in, with Silicomolybdate.....	269	Determination in Egg Powder.....	628
Tungsten Determination in.....	45, 607	Distilled, of High Purity, Column for Continuous Production of....	748
<b>Still.</b> See Distillation.		-Ethyl Ether-Benzene-Ethanol Solutions Analyzed.....	432
<b>Stopcock</b> Lubricants.....	415	Evaporator for Determination of Total Solids in.....	120
Styrene, $\alpha$ , $\beta$ -Dimethylstyrene Determination in Presence of.....	20	Ion-Exchange Adsorbers for.....	616
Styrene, $o$ -Xylene Determination in.....	751	Magnesium Microdetermination in, with 8-Hydroxyquinoline.....	595
<b>SUGARS</b>		Phenol Determination in, by Gibbs Method.....	694
Analysis, Lead Subacetate Specifications as Reagent for.....	281	Reservoir for Hot Distilled Water.....	201
$d$ -Galactose Determination by Selective Fermentation.....	28	Silica Determination, Dissolved in.....	612
Maltose Determination in Presence of Glucose.....	582	Valve of Constant-Level Float Type for Filling Bottles.....	201
Microdetermination, Sichert and Bleyer Reagent Modified for. (Correction, 693).....	537	Vapor Permeability of Organic Films.....	686
Phosphomolybdic Acid Color Reaction with.....	637	(See also Humidity and Steam.)	
Sulfanilic Chloride, Acetyl-, Analysis by Karl Fischer Reagent.....	517	Wax Determination in Cotton Fiber by Alcohol Extraction.....	745
<b>SULFATE</b>		<b>WEIGHING</b>	
Determination in Cellulose Nitrate and Other Esters.....	391	Bottles, Device for Rapid Closing of.....	579
Microdetermination by Benzidine Sulfate Precipitate.....	536	Buret, Modified Bailey Type of.....	357
<b>SULFUR DETERMINATION</b>		Funnels.....	133
in Brass and Bronze by Combustion.....	349	Weights Corrected to Vacuum, Nomograph for.....	275
in Butyl Rubber, Total and Combined.....	98	Wij's Solution. See Iodine Number.	
in Feeds by Nitric and Perchloric Acid Digestion.....	630	<b>WOOD</b>	
Sulfur Dioxide Determination in Dehydrated Foods.....	153	Cellulose. Viscometric Chain Length in Triton F.....	683
Sulfuric Acid, Hydrocarbon Analysis by Extraction with. (Correc- tion, 727).....	558	Furfural Determination by Iodine Method in Hydrolyzed Liquors from.....	319
Surface Area, Apparatus for Measurement of.....	398	(See also Pulp.)	
Sweet Potato. See Potato.		<b>X-RAY</b>	
<b>TALL OIL</b> , Iodine Number Method for.....	371	Camera, Funnel for Filling Capillaries of.....	343
Tannin Colorimetric Method for Beryllium and Aluminum.....	598	Crystalline Materials Determined by Diffraction Patterns.....	95
Tenderness of Dehydrated Fish Determined in Instrument for Measur- ing Texture Changes.....	638	Phenyl Isocyanate Derivatives of Alkylated Phenols Identified by..	304
Ternary System Acetone-Benzene-Water Analyzed.....	499	Powder Diffraction for Chemical Analysis.....	209
Tetraethyllead Determination in Gasoline.....	4	Xanthone Spray Residues, Colorimetric Analysis of.....	35
Textile-Finish Identification Stains.....	274	Xanthophyll Determination in Plants.....	438
<b>THIAMINE DETERMINATION</b>		$o$ -Xylene Determination in Styrene Recycle.....	751
in Beef Muscles. Comparison of Methods.....	116	Xylene in Extraction of Oil and Vitamin A from Shark Liver.....	126
in Pharmaceuticals. Rat-Curvature, Thiochrome, and Fermen- tation Methods.....	476	<b>YEAST</b> Growth or Fermentation Methods for Thiamine Determina- tion.....	576
by Thiochrome Method.....	572	Yeast Microbiological Determination of Pantothenic Acid.....	67
by Yeast-Growth, Yeast-Fermentation, and Thiochrome Methods....	576	<b>ZEISEL</b> Method. See Methoxyl.	
Thiochrome. See Thiamine.		Zeo-Karb as Exchange Adsorber, Quantitative Separations with.....	615
Thiosulfate Washers in Alkoxy Microdeterminations.....	207	<b>ZINC</b>	
Timing Siphon.....	592	Alloys, Microdetermination of Copper, Lead, and Cadmium in....	71
<b>TIN</b>		Benzoin as Fluorescent Microreagent for (Correction).....	102
Alloys, Antimony Determination in.....	448	Determination in Cyanide Brass-Plating Baths.....	165
Determination by Iodometric Method.....	555	Determination with 8-Hydroxyquinoline.....	256, 460
Microdetermination with Silicomolybdate.....	269	Determination in Magnesium Alloys.....	703
Separation from Copper in Electrodeposition Apparatus.....	532	Determination in Naphthenate of Zinc.....	194
<b>TITRIMETRY</b>		Determination by Titrimetric Method.....	758
Chlorometry as Procedure for Microanalysis.....	206	Microdetermination by Fluorescent Turbidity Measurement.....	281
Conductometric Apparatus for.....	591		
Microburet for.....	475		
Rack for Semimicroanalyses.....	346		
Volume Measured Precisely in.....	50		
Tobacco, Nicotine and Normicotine Determined in.....	86		
Tobacco, Normicotine Identification in.....	377		
Torch, Micro.....	142		
Toxicity of Acrylonitrile in Air, Microtests for.....	63		
Triton F (Dimethyldibenzylammonium Hydroxide), Viscometric Chain Length of Wood Cellulose in.....	683		
Tung Oil Estimation as Adulterant.....	511		
Tung Oil, Heat-Bodied, Analysis of.....	90		
Tungsten Determination Gravimetrically with Anti-1,5-di-( $p$ -methoxy- phenyl)-1-hydroxylamino-3-oximino-4-pentene.....	45		
Tungsten Determination in Steel Spectrographically.....	607		















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